

Poor press losing argument

True to form, last month's meeting of UNCTAD was another failure. The rhetoric of the new economic order made developing countries miss an opportunity to win the only prize worth having.

THIS year's meeting of the United Nations Conference on Trade and Development (UNCTAD) has, after all, been a sad disappointment. The statement put out at the closing session in Belgrade last Saturday, from which the United States "dissociated" itself and about which Britain, Japan and West Germany expressed "reservations" (diplomatic code for the same thing), amounts to a repetition of the now familiar demand by developing countries for what is called a new economic order — more credit for countries in financial difficulties, guaranteed prices for commodities produced by developing countries and a general commitment by the rich nations to share their wealth more equitably than at present. Both kinds of governments at the meeting in Belgrade seem to have missed the opportunities that seemed apparent just four weeks ago (see *Nature* 16 June, p.560).

What has gone wrong? There is one overriding cause of the failure at Belgrade — the insistence of the developing countries on an agenda broader than the delegations from the industrialized countries were willing or even competent to discuss. While there may be something to be said for discussions between the rich and poor nations on these general questions, such as that held at Cancun in Mexico two years ago, UNCTAD is not the place for them. Moreover, by choosing to follow such a broad agenda, the developing countries have diverted attention from what should have been their chief concern — to put a stop to the steady encroachment of protectionism in trade on their own freedom to work and pay their way out of trouble.

The circumstances are well known. The world recession that began in 1973 has decreased the export earnings of the oil-importing states precisely when their import bills were by the same token increased. The appendix to the Brandt Commission's report published earlier this year estimated that the trade deficits of the oil-importing states amounted in 1981 to \$88,000 million — a huge sum only partly offset by official aid and by loans from international lending institutions such as the International Monetary Fund. The result is that the poorest states are now saddled with an accumulated debt of \$85,000 million to the commercial banking system, an amount that would certainly have been much larger if more credit had been available but whose cost has been made more onerous as industrialized nations have used high interest rates as a weapon in their struggle against inflation. It is forgivable that the developing countries should think this problem would go away if there were more credit available (and the resources of the International Monetary Fund will be increased by a half some time next year). But no amount of extra credit could be more than a stop-gap — a device for increasing still further what the poor countries owe and must in due course repay.

This is why the immediate but also the long-term need is that the poor nations of the world should be enabled by their own efforts to earn more by their exports. And that is why removal of restraints on the trade of the developing countries should have dominated the proceedings at Belgrade. With a little ingenuity, indeed, the developing states should have been able to shame their richer partners into more intelligent policies. The way in which most rich countries support their domestic agriculture by subsidies to farmers and by tariffs, quotas or the flat prohibition of imports is a scandal — and a cause of needlessly high prices to their own taxpayers. So is the common practice of protecting manufacturing industries by means of tariffs on simple manufac-

tures (but not on the commodities from which they are made): it prevents developing countries from making their own way in the world by adding value to their primary commodities, selling cloth and not just cotton, for example.

In these and other ways, the rich nations of the world restrain the volume of international trade, help to postpone the return of prosperity and in the process create especially cruel burdens for the developing countries. Delegations from the poor countries at the Belgrade meeting would have been on firmer ground if they had argued the folly of this mindless protectionism. They might have pointed to last week's little irony — the announcement by the United States Government of a complaint to the General Agreement on Tariffs and Trade (GATT) that Japan is breaking the rules on agricultural imports coinciding with various congressmen's complaint that Japan's decision to end next April a voluntary quota on exports of automobiles to the United States is a "second Pearl Harbor" (Congressman John Dingle). Nobody pretends that automobiles are at the top of the list of the poor nations' frustrated exports, but all steps that artificially reduce the volume of international trade must ultimately hurt them.

But how can the rich nations be persuaded to abandon protection when their own domestic problems, unemployment for example, are more serious than for many years? The conventional wisdom in places as different as Washington, Brussels and Tokyo is that prosperity must first return. The flaw in that argument is that trade protection is a short-term way of saving jobs in industries which are no longer competitive and which must be paid for not merely in the higher prices consumers pay for domestically produced goods but in the inhibition of industrial change that it engenders. In the long run, now-rich countries will be as much harmed by perpetuating an outdated division of labour between themselves and the poor countries of the world as will be the developing world itself. This is what the group of 77 should have been saying in Belgrade. To have made the argument effectively, it would have had to acknowledge that rich governments would have to increase domestic expenditure on the consequences of more rapid change — and that more liberal trade will not help those among its own members that so mismanage their affairs that they have nothing to export. To have failed to make the argument is a sorry loss of an opportunity that may not recur for many months.

Keeping hands clean

Academics should complain less and worry more about rules against conflicts of interest.

NEXT week, the California Fair Political Practices Commission will once again take up the question of financial disclosure requirements for University of California faculty. It is now a little over a year since the commission reversed its long-standing policy of exempting the university from the state's political reform act and demanded that, like other state employees, professors should disclose their personal financial stakes in official decisions, particularly their links with the corporate sponsors of their research. The year's experience now to hand shows that the system is working; it also shows that the concern that led to the new system was not exaggerated. Unfortunately, that message has yet to sink in



with the many faculty members who continue to complain about invasion of privacy, paperwork and obstacles to industrial cooperation. It is time for them and a still reluctant University of California administration to come to grips with reality: the commission is past discussing whether the rules are necessary (they are) and has turned now to asking whether they are sufficient. To ignore this reality is to continue arguing yesterday's case.

Under the rules adopted a year ago, faculty members are still shielded from the full force of the state political reform acts. While professional employees elsewhere in the government must file annual financial disclosures of their interest in any corporation likely to be affected by their official actions, faculty members are required only to reveal interests in a research sponsor, and only when submitting a new grant to the university for approval. Statistics up to the end of May show 4,340 statements filed, 210 of which indicate some financial interest (consulting, investment or service in some official capacity) in the sponsor. Approximately half were trivial, membership of the board of a sponsor such as the American Heart Association for example. The university and the commission have drawn up a list of such charitable organizations and are exempting their grants from review by the university committees set up to examine cases where financial interests are reported. The commission may next week dispense with even the filing requirement for such grants.

But another 100 or so positive responses involved private sponsors and some of these were far from trivial. And in three cases, the review committees (which include faculty, staff and students) have insisted upon substantial changes in the terms of a grant before approving them. Two recent instances at the Berkeley campus are especially instructive. Professor Milt Schroth of the plant pathology department was not allowed to accept an \$89,000 grant from Advanced Genetic Systems, a company in which he owns stock and for which he serves as a scientific adviser. Schroth agreed to step aside as principal investigator, although he will still be involved in the research, which concerns plant-root colonizing bacteria. In the other case, the committee insisted on the removal of a restriction clause in a contract from seven minerals companies to Professor H. Frank Morrison that would have blocked publication of computer codes used in the research unless the sponsors gave their permission. Despite Morrison's increasingly irate complaints — which culminated in a letter to the university's chancellor accusing the committee of pettiness and vindictiveness and complaining that such action would only "senselessly antagonize sincere supporters of university research" — the committee held its ground and left no doubt that it would approve the deal only if the clause was dropped. It was.

In the light of the result, Morrison's complaints have a hollow ring to them; likewise, the grumbling heard from the university administration about paperwork and "only" turning up three doubtful cases in a year are less than convincing. The system works; fairness and academic freedom have been served and research support has not ultimately suffered. Instead of flogging a dead horse, the academic community in California — and elsewhere for that matter — would do well to accept limited financial disclosures and scrutiny of research arrangements for conflicts of interest as an accomplished fact, and expend some serious thought on some of the more subtle issues raised by the new university-industry ties. Professor Leon Wofsy of Berkeley has pointed out several of these: the growing tendency to measure success by the ability to swing deals, rather than to publish good research; the effects of patents and licensing arrangements on research and institutional decisions at the university; and the subtle subjugation of peer review to administrative fiat when large sums are involved. (Wofsy tells of being called recently, along with a dozen other members of his department, to a meeting with the dean to hear of the intention of one company to support half a dozen research projects with grants of \$30,000–\$40,000 each. In these no doubt special circumstances, peer review took the simplified form of the dean's nomination of those who would be invited to apply for grants in a competition with a 50:50 chance of success.) Plainly, sheer financial conflict of interests is just the tip of the iceberg. □

Technology and hanging

The clamour to bring back the death penalty in Britain overlooks new technological opportunities.

ONE of the predictable consequences of the British Conservative Party's massive parliamentary majority in last month's general election is the resurgence of political interest in capital punishment, which ceased to be a criminal sentence in 1965. Since then, there have been three occasions when the House of Commons has voted to confirm the disuse of the death penalty. The new development is that many of the new members of the House of Commons are said to have won their chance to represent the Conservative Party at the election by promising local selection committees to support the hangman's return in the "free" vote on the subject which the government foolishly promised in its election manifesto.

There are several dangers in this development, not the least of which is that the Conservative Government's hope to be known as a go-ahead high-tech government ("cabling Britain" and all that) will be tarnished if it is now seen to derive its political power from people attached to an old-fashioned technology such as hanging. Although the secrecy surrounding prison executions has allowed the boast that "British hangmen are best", it is unfortunately known that the technology leaves much to be desired. The procedure is intended to break a person's neck, but its effectiveness depends to some extent on the angle at the bottom of what is called "the drop" between the plane of the rope and the dorsal-ventral plane through the spine. This is why a good hangman must be an agile fellow, able to leap from his scaffold to hang on the legs of a subject not killed outright. Can even the newest members of the House of Commons ask their government to administer such an empirical technology?

Unfortunately, there is little help to be won from experience elsewhere, even in the United States. The patchy return of capital punishment in various states has revealed that even the once widely practised technique of electrocution has its defects, perhaps because the untrained prison guards are out of practice, while the outwardly more up to date technology of a massive intravenous infusion of barbiturates followed by toxins has been impeded by unskilled people's difficulties in finding veins as well as by problems with piping and the like. It would, however, be a serious setback for a government pretending to be technologically up to date to have to fall back on an old-fashioned method. The hanging lobby in the House of Commons must quickly say which technology it wishes to be practised.

It must also come to some kind of a conclusion about publicity for capital punishment. The promises that have been given to constituency parties about the restoration of the death penalty appear to have sprung from the demand that "tough measures" should be seen to be taken on "law and order". Especially because the present government has pinned its faith on the engineering of a communications revolution, it is only natural that it should be anxious to demonstrate its practice of the toughness asked of it by using the new communications technology to make executions discreetly public, perhaps by compelling the operators of the cable television systems soon to spring into being to carry video recordings of them. The snag, of course, is that the "hangers" in the new parliament include many people who also carry a torch to outlaw obscenity on public television. This is another issue on which the hanging lobby should make up its mind.

That the restoration of the death penalty would have little effect on, say, the incidence of murder in the United Kingdom (where most murders kill people with whom they are emotionally entangled), and would probably complicate the problem of terrorism by making martyrs of those terrorists who were caught and convicted, will no doubt dominate the debate in the House of Commons now being planned. The dangers inherent in the plan to bring back an old-fashioned technology are being overlooked. The danger that the hanging lobby may prevail numerically is, fortunately, more clearly perceived. □

UK research councils

Demand for cuts angers science council's staff

AS IF ITS budgetary problems were not enough, the UK Science and Engineering Research Council (SERC) has also to consider its response to a recent review of its activities by the British Government's cost-cutting unit. The draft report, which has not yet been published, claims that savings of £3.3 million could be achieved on capital costs and £450,000 a year on running costs. Suggested measures include the loss of 50 jobs, the sale of Herstmonceux Castle (which forms a substantial part of the Royal Greenwich Observatory (RGO)) and the possible merger of the Royal Observatory Edinburgh with RGO. A staff response to the report maintains that it is based on insufficient consultation, incorrect assumptions and a lack of consideration for wider scientific issues.

The unit that carried out the scrutiny was set up by Sir Derek Rayner at the instigation of the Prime Minister, Mrs Margaret Thatcher, to cut a swathe through the tangled undergrowth of public expenditure with the aim of cutting costs and increasing efficiency. It has prepared draft reports on the Medical, Agricultural and Natural Environment Research Councils as well as SERC. As part of its procedure, one or more members of the councils joined their respective scrutiny teams which also included representatives of the Department of Education and Science and the Management and Personnel Office. Responses have been or are being prepared by both the staff and management sides of the councils, and the collected reports will then be sent to Sir Keith Joseph, Secretary of State for Education and Science.

Financially, the most significant of the recommendations made in the report on SERC concern the sale of Herstmonceux Castle and of 95 houses owned by SERC and rented to people working at the Rutherford Appleton Laboratory. Between them these recommendations would, according to the report, result in a capital saving of £2.55 million, together with decreased recurrent costs at the castle. (The report points out that under current regulations the proceeds of the sale would have to be paid to the Treasury and advocates a change so that the council can benefit more directly.) Both recommendations are attacked in the reply from SERC's staff.

The castle at RGO houses a substantial archive of astronomical records and the observatory's library, meeting rooms, etc. The Rayner unit maintains that the archive is "little used" and that it would be more appropriately kept by the Public Records Office, while other facilities could be accommodated by constructing an extension to the other main portion of the obser-

vatory, the West Building complex which accommodates most of the staff.

In its reply, the staff maintains that the Rayner unit has, through lack of consultation, under-estimated the importance of the archives for astronomical research, that a substantial number of people make use of them and that the Public Records Office itself is "firmly of the opinion" that RGO should house the archives.

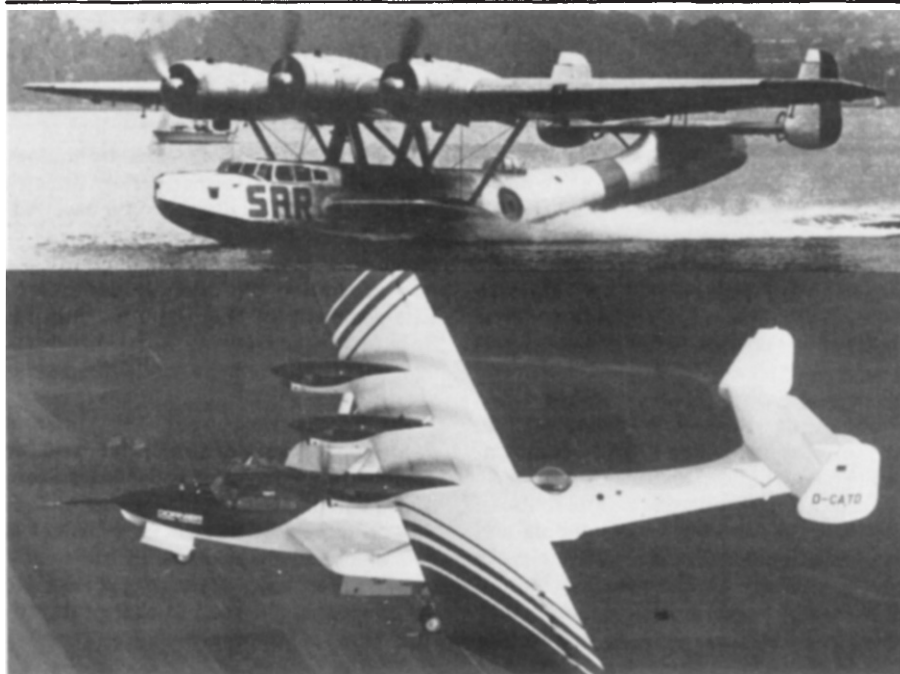
Other recommendations concerning RGO's on-site facilities have infuriated the staff. RGO's role is increasingly related to operation and support of observatories at such remote sites as La Palma in the Canary Islands. "The nature of the work at the observatories", says the report, "has therefore changed as the on-site facilities have become obsolete." The staff replies that here the Rayner unit lacks relevant experience and has stepped outside the bounds of its remit. Surprisingly, the report does not mention the development at RGO of the much-publicized remote control of telescopes which is expected to increase the number of observers visiting the observatory.

The Ray0028-0836/83/270002-02\$01.00 lations for RGO suggest that, by selling the castle and some of the land, £1.1 million capital and

associated running costs could be saved. The report also recommends that the implications of a merger of RGO and the Royal Observatory Edinburgh should be assessed by SERC before April 1984. "This", says the staff reply, "really does smack of a preconceived idea being ventilated and is a problem of such complexity that the inspection team itself has merely asked the question of council . . . Our attitude, from the RGO point of view, is that the report can be summarized as arriving at very drastic conclusions after very minimal consultation."

Other recommendations by the Rayner unit appear to have fewer scientific ramifications. The 95 houses used by SERC as accommodation at the Rutherford Appleton Laboratory have a freehold value of £2.5 million, the rents and costs such as maintenance being roughly balanced. SERC is not paying for loans on these houses, so that the arguments concern such imponderables as the value of the houses in helping to recruit staff. The remainder of the recommendations concern details of maintenance, building supervision and stores which would, according to the report, save £448,000 a year in running costs and cut 54 posts. Many of these are opposed by the staff.

The SERC management refuses to discuss the report in detail, but expects to have prepared its response by the end of July. It is, however, likely to be sceptical of the major recommendations. Whether the issues raised will ever be publicly discussed is not at this stage clear. **Philip Campbell**



THE Dornier aircraft company of West Germany is evoking old memories of magnificent flying machines by toying with this 20-40 seater flying amphibian, a modest redesign of the Second World War flying boat the Do24. The old aircraft, pictured above in service with the Spanish air force, proved to have a superb hull-form, says Dornier, a form that allowed it to land and take off in very rough seas. This technology has not improved since the war — so Dornier engineers have retained the hull in a new, experimental model (the Do 24 TT, below). But they have provided a much more efficient (supercritical) wing, benefiting from modern aerodynamics. The target market is island transport, where helicopters are now much used, or long-range offshore patrol, policing and rescue.

Acid rain

Demands for action multiply

Washington

BUCKING the Reagan Administration line that too little is known about acid rain to take regulatory action, the White House Science Office's own panel of experts last week called for "meaningful reduction" in sulphur dioxide emissions and warned that delay could cause irreversible damage to the environment. "Actions have to be taken despite incomplete knowledge", the panel concluded, and suggested that initial "least cost" steps might include use of low-sulphur coal in summer (when wet deposition of sulphuric acid reaches a peak in North America), intensive coal washing and imposition of emission controls on non-ferrous smelters.

The White House panel, headed by William Nierenberg, director of the Scripps Institute of Oceanography, was formed to review the scientific documents produced by US-Canadian working groups set up under the "memorandum of intent" signed by the two countries. The job was to have been assigned to the National Academy of Sciences, but when an academy report in 1981 called for an immediate reduction of 50 per cent in acid deposition, Reagan officials concluded that the academy was too biased and mustered its own experts.

The administration decided at the same time not to support a second academy study, of atmospheric processes and deposition. The academy had its revenge last week when it released that study (financed by private foundations) the day after the White House panel issued its findings.

The chief conclusion of the academy group is that the conversion of acid precursors (sulphur dioxide and nitrogen oxides) to acids in the atmosphere is a linear process, when averaged over large areas and periods of one year. Thus a 50 per cent reduction in emissions over the entire eastern half of the United States will yield a 50 per cent reduction in deposition. These findings contradict the conventional wisdom — based largely on European monitoring data — that the response would be nonlinear, with perhaps even substantial cuts in emissions failing to give a commensurate pay-off in deposition.

The academy group was careful to stress, however, that current understanding and modelling is woefully inadequate to make any more refined predictions. The effect of emission controls in a particular area (such as the midwest) on deposition in a receptor area (such as the Adirondacks) cannot be evaluated, the group said.

Although Nierenberg said he hoped his panel's findings will become an administration statement, a White House official appeared to hedge when asked how the findings will be used. He skirted the issue of regulation, saying that was up to William Ruckelshaus, administrator of the

Environmental Protection Agency, to decide, and emphasized instead the panel's recommendations — which will be out shortly — on research priorities in acid rain.

Meanwhile, two bills before Congress would order action. Representative Henry Waxman (Democrat, California) and Gerry Sikorski (Democrat, Minnesota) have proposed a gradual nationwide reduction in sulphur dioxide emissions; and the Senate Environment and Public Works Committee has already approved a measure that calls for reductions in the midwestern and western states only.

Jack Calvert, of the National Center for Atmospheric Research, who chaired the academy group, said its results did not necessarily support either of these ini-

atives, and that any regulatory decision had to take into account not only scientific findings but also economic and political factors that the academy panel was not competent to assess. But he did say that its conclusions mean that "we'll guarantee an effect" if emissions are reduced — which is more than has been said until now.

The academy group's conclusion on this point was based largely on data collected at Hubbard Brook in New Hampshire, which has been intensively studied for the past 13 years. The ratio there of deposited sulphate to nitrate ions has closely paralleled changes in the emission rates of the corresponding precursors in the eastern United States. The group suggested that contradictory European data may reflect a difference in meteorology or climate, or may be due to changes in analytical and sampling techniques and laboratories over the period of data collection in Europe.

Stephen Budiansky

US cooperative research

Antitrust restraint on way out

Washington

HIGH technology companies in the United States expect soon to be allowed to form collaborative research and development ventures with their domestic competitors without fear of violating the antitrust laws. The Senate began hearings last week on three bills that would respond to longstanding industry complaints that antitrust legislation has tied the hands of American companies seeking to respond to the technological challenge from European and Japanese rivals.

Inspiration for the measures coincided with the recent formation of the Microelectronics and Computer Technology Corporation (MCC) by 13 high technology firms. Headed by former deputy director of the Central Intelligence Agency, Admiral Robert Inman, MCC is expected to play a leading part in the development of a new generation of American computers. Establishment of the ventures, possible only after navigating tortuous legal obstacles, has become a model for similar ventures.

In hearings last week Mr Malcolm Baldrige, Secretary of Commerce, said the administration regarded collaborative ventures such as MCC as highly desirable as they promoted the efficient use of technical personnel and enabled small firms to collaborate in ambitious research projects. He acknowledged the need to change the antitrust laws to encourage joint ventures.

Baldrige claimed that the United States's major trading partners had already invested heavily in collaborative efforts, and cited Japan's ten-year joint research programme to create a fifth-generation computer. The European Economic Community, too, had exempted several joint research agreements from its antitrust restrictions.

Arguing that recent attempts by the Department of Justice to explain how the antitrust laws could be circumvented in some cases had not eased the fears of many companies, Baldrige disclosed that the administration was preparing a package of legislative reforms which would exempt joint research and development from all private antitrust actions, and from all government actions for damages based on activities that were part of a research programme that had been publicly disclosed. But the government would still be able to seek an injunction to halt any actions thought to be harmful to competition.

Although the administration package goes further than many of the measures introduced in Congress, it has come under considerable criticism from sponsors of the congressional legislation and from the industries that would benefit. Senator Charles Mathias (Republican, Maryland) warned Baldrige that by coupling the research and development exemption with more extensive changes in antitrust law, the administration might lose congressional support. Admiral Inman also warned against overloading the modest bill with too many controversial reforms.

In addition to its research and development exemption, the administration bill would end the "triple damage" provisions which have been the cornerstone of antitrust legislation for many years. While Congress is expected to support the exemption, many congressmen are likely to balk at a radical change in the general legislation. Senator Mathias described triple damages as one of the law's "historic bastions". And Senator Howard Metzenbaum (Democrat, Ohio) claimed that the disappearance of triple damages would "decimate" the antitrust regulations.

Peter David

French research

Trouble brews in the ranks

NEW regulations demanding more teaching from lecturers (for the same pay) and a leak of the long-awaited regulations which appear to restrict the career hopes of technicians were causing French university researchers and laboratory technicians to feel distinctly unhappy at the end of last week. Technicians in a major research area at Grenoble, traditionally the thermometer of feeling in the research ranks, immediately went on protest strike for an hour on Friday. And in Paris, the head of a scientific department in the university said he would have to "cheat" the proposed new teaching regulations, announced by the government last Wednesday, if his research was not to suffer. If the government gets its way, the new regulations will take effect this autumn.

In Grenoble, the technicians had learned that the new rules affecting them — unpublished but now before the Prime Minister, Pierre Mauroy, for approval — were "completely different" from the grading and salary agreements that had been negotiated over two years with the previous research minister, Jean-Pierre Chevènement.

According to the director of a Grenoble physics laboratory, the unions seem to have been treated "very badly" by the new minister, Laurent Fabius. They had assumed that the regulations before Mauroy were the ones negotiated; now it seems they are not, and that key problems dealt with in the Chevènement rules — such as the present lack of promotion or salary advancement for good work once a technician has reached mid-career — remain unresolved in the Fabius version. Indeed, regulations proposed by Pierre Aigrain, the science minister of the previous (conservative) president, Giscard d'Estaing, might have been more acceptable, it is being said in Grenoble. "As a director of a laboratory, I really hope the government will restart negotiations", the Grenoble physicist said last week. Otherwise, research in Grenoble and throughout France seems to be threatened with disruption by strikes.

Among university lecturers and professors, tension now centres on the new teaching demands of four hours of lectures per week for 32 weeks compared with three hours for 25 weeks at present. At the same time, the institution of paid *heures complémentaires* — essentially overtime teaching — which cost the ministry of education FF 100 million (£8.7 million) a year to finance, is to be abolished. Student numbers are also to increase.

The result is complicated, and will vary greatly from discipline to discipline. In the humanities, for example, where most *heures complémentaires* are taken, the net effect may be to reduce lecturer's take-home pay. But in the hard sciences there is little tradition of teaching *heures comple-*

mentaires, lecturers preferring to devote the time to research. So in such disciplines the net increase in teaching load seems likely to be compensated by a reduction in research time. An increase in the number of statutory managing committees in university departments from two to three, defined in the new higher education law (which has passed its hurdles in the National Assembly and goes to the Senate in September) is likely to have the same effect: to reduce the time for research.

Unless, that is, people feel inclined to fool the system, like the University of Paris professor, who will be over-claiming on administration time and counting as teaching time "any time when there's a single

student in my laboratory". This, he admits, is not the best solution, but nothing else — other than new regulations — would allow him time for research. He — like many of his colleagues — is bitter at the contrast in treatment between the universities and the *grandes écoles*, the tiny engineering schools where France trains its elite but where with few exceptions little research is done. The *grandes écoles* are virtually untouched by the higher education law and the new regulations.

The consequence has been a sharp swing of mood among French scientists, especially those whose votes helped to bring in the present government in May 1981. In Chevènement's time, there was great excitement and, as the physicist said last week, "people in research were told they were the best. But now," he added, "all that seems to have gone." **Robert Walgate**

French nuclear tests

Invitation to Muraroa on table

Canberra

A SPECIAL envoy of President François Mitterrand of France has invited each independent South Pacific state to send a scientist to the French nuclear testing site at Mururoa Atoll. The invitation marks a change in policy by the French Government which has hitherto been insensitive to protests at the testing of its "independent nuclear deterrent" in the South Pacific.

M. Régis Debray, personal adviser to the President, was sent on the mission to defend nuclear testing and to defuse tension in the region before the South Pacific Forum meeting in Canberra which has on its agenda the nuclear tests and independence for French New Caledonia. Debray's trip follows soon after the Australian Prime Minister, Mr Bob Hawke, protested in person to the President and suspended a shipment of uranium bound for France from Queensland, in early June. The fate of the whole export contract with France is now in doubt. Although there is little doubt that the French were planning some concessions of the kind offered by Debray before the Hawke visit and although France is not dependent upon Australian supplies of uranium, the top-level meeting in Paris may have hastened the French gesture. Certainly France regards suspension of uranium shipments as "an unfriendly act".

The French offer to open the site to inspection, strictly a one-off affair, does not in fact amount to much. If taken up by South Pacific countries — the invitation extends to Fiji, Papua New Guinea, Vanuatu, Western Samoa and Tuvalu as well as Australia and New Zealand — the delegation will visit the site only after the testing is over for the year. Furthermore, Debray says "the French Government draws an absolute distinction between inspection and monitoring", which implies

that the scientists may be severely restricted in what they might do. It is not clear at this stage if the scientists may take along equipment nor how long they may stay. Conceivably, the delegation may be able to tell whether the atoll is sinking, cracking, or both, under the strain of the testing, as has been alleged by anti-nuclear groups. However, all previous geological, meteorological and radiation data, which would be needed for comparison, have been compiled by the French.

New Zealand, which has long been pressing for an observer at the tests, has taken up the invitation with alacrity, and Dr Hugh Atkinson, a radiologist of the New Zealand National Radiation Laboratory, has been asked to head the delegation of scientists. As a further concession to New Zealand, some French technical information, denied in the past, will be made available to the laboratory.

Australia's position is rather more complicated, and it has not so far taken up the French offer for fear it may be interpreted as a tacit acceptance of the tests, although the federal cabinet is expected to discuss the matter soon. The government has to appease a strong, restless, anti-nuclear left-wing faction in caucus that has been less than happy with the recent granting of uranium mining contracts to two companies in face of promises that no new uranium contracts be entered into. In addition, Australia will push for a nuclear-free zone in the South Pacific in the coming meeting of South Pacific Forum countries, and vigorously campaign for a comprehensive test ban treaty in the United Nations.

Against this backdrop, the cabinet may well try to extract more concessions from the French, using export contracts with France as a lever, such as asking for an increased number of scientists and greater freedom to conduct experiments.

Vimala Sarma

Dioxin lawsuits

Agent Orange in the courts

Washington

AN attempt by the American Medical Association (AMA) to calm public fears about the health effects of dioxin appeared to backfire last week when an AMA spokesman was forced to admit in a congressional hearing that a resolution dismissing its dangers had been "imprudent". The resolution, adopted two weeks ago by the association's House of Delegates, had accused the media of launching an irrational "witch hunt" against dioxin and said the substance appeared to pose no serious health risk to humans.

Dr John Beljan, an AMA spokesman, said that while the resolution had been poorly worded, AMA stood by its claim that there was still little evidence to support allegations that exposure to dioxin had been responsible for the widespread health defects attributed to it. AMA intends to publish later this year a report summarizing the state of the scientific evidence.

An estimated 65 federally-funded dioxin studies are now in progress, but their findings are not expected to receive a dispassionate reception. The scientific evidence is being rapidly converted into legal evidence as part of an emotive confrontation between some 15,000 Vietnam veterans and the chemical companies that manufactured Agent Orange, the herbicide used as a defoliant during the Vietnam war.

The veterans blame dioxin within 2,4,5-T (a component of Agent Orange) for a variety of illnesses including cancers and birth defects. They are suing the chemical industry for damages that could cost hundreds of millions of dollars if the court agrees to treat the case as a class-action suit. In that case, all 2.5 million veterans and their families could claim compensation for health defects they attribute to Agent Orange.

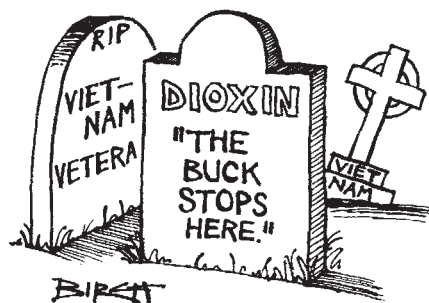
A trial scheduled to begin last week has been postponed for at least another year to give the veterans and the chemical companies time to accumulate more evidence. Papers already filed by the veterans have been sealed by the New York court hearing the case, but enough details have been leaked to make it clear that some of the manufacturers were aware of the health risks of 2,4,5-T several years before spraying with Agent Orange ended in 1970.

How much the chemical companies knew about dioxin contamination when they manufactured Agent Orange has become a key issue since the companies being sued decided to make a "government contractor defence". If the companies can prove that the federal government knew as much about dioxin as the manufacturers, and that Agent Orange was manufactured to government specifications, the companies will be covered by federal immunity from liability.

Arguments at the trial will therefore con-

centrate on two issues: did the chemical companies suppress their knowledge about dioxin contamination in Agent Orange in order to protect the lucrative 2,4,5-T industry; and can exposure to Agent Orange have caused the wide range of health defects which the veterans ascribe to it?

Leaked correspondence between the chemical companies suggests that at least one manufacturer, Dow Chemical Company, was sufficiently worried in 1965



about the high levels of dioxin to have summoned its competitors to a meeting to share its findings.

The decision to postpone the trial for at least another year means the court will have access to considerably more scientific evidence. And judging by the reception last week of the first Agent Orange study, the scientific evidence will be as contentious as the question of liability. Mr Victor Yannacone, who initiated the veterans' action against the companies, dismissed as "worthless" the finding of an Air Force study of more than 1,200 personnel who had been directly involved in the "Ranch Hand"

operation during which Agent Orange was sprayed from US aircraft.

Dr Alvin Young, an Air Force medical adviser, said a mortality study had revealed no difference in the death rate of Ranch Hand personnel and other veterans who had not been exposed to the herbicide. Although Ranch Hand personnel handled large quantities of Agent Orange without wearing protective clothing, only four deaths from cancer had occurred and there were no reports of soft-tissue tumours. Yannacone claimed that the Ranch Hand study was statistically inconsequential.

In congressional evidence last month, however, the Veterans Administration deputy chief medical director, Dr William Jacoby, said he was confident that the range of studies now under way would provide "scientifically acceptable answers" about the health effects of Agent Orange. He cited eight major investigations that are expected to yield results within the next year. They include a mortality study of 60,000 deceased veterans, a study of identical twins where only one brother served in Vietnam and an investigation of a possible link between service in Vietnam and soft-tissue sarcomas.

Although the Veterans Administration is leading the federal research effort in Agent Orange, it is regarded with suspicion by many veterans because it is also the agency that would be responsible for compensating victims of service-related illnesses. Bowing to pressure from veterans, Congress has directed that a major epidemiological study of Agent Orange be transferred to the Centers for Disease Control, Atlanta which are also conducting a study of birth defects in the children of Vietnam veterans, with publication expected early next year.

Peter David

Ne'eman's too broad Israeli horizon

ISRAEL could be short of 5,000 engineers and 8,000 engineering technicians by 1992, according to Dr Yuval Ne'eman, the minister of science and research.

Reporting on his ministry's first year of activity, Dr Ne'eman told the Knesset last month that the expected shortfall in the training of engineers could seriously delay Israeli plans to develop science-based industry. The main problem, he said, is a lack of lecturers, with even such a prominent institution as the Technion having difficulty in filling all its appointments.

Science-based industry is widely acclaimed as Israel's key to future prosperity, although Dr Ne'eman himself believes that in the long run it will be the export of know-how rather than of technology that will become increasingly important. He is a keen supporter of the proposal to develop "Region 2000", the projected "science area" to be based on Karmiel in north-west Galilee, which would create an estimated 10,000 jobs in high-technology industries

and perhaps another 10,000 in other sectors.

The official proposals from the ministry for trade and industry for Region 2000 stressed that it should "promote constructive coexistence" between Jews and Arabs in the area, "to the benefit of both communities and without the strains caused by competition for available arable land". Dr Ne'eman, however, is well known for his "hawkish" politics, and in the debate on his minister's report was attacked for supporting schemes over which public opinion is, to say the least, divided. These include plans for science cities at Ariel (Samaria), Katzim (on the Golan Heights), Krayat Arba (Hebron) and also the southern route for the Mediterranean-Dead Sea canal, crossing the Gaza Strip. According to Mr Shevah Weiss of the (opposition) Alignment, no fewer than 8 of the 14 projects emphasized by Dr Ne'eman involved sites outside the pre-1967 frontiers.

Vera Rich

US electron accelerator

Designers dispute over machine

Washington

WITH the recommendation by a government advisory panel earlier this year that the contract for a new electron accelerator be awarded to an inexperienced consortium of south-eastern universities, and with extraordinary efforts by the loser, Argonne National Laboratory, to have that recommendation overturned, feelings have been running high among high-energy physicists. So when a respected accelerator designer from Fermilab wrote to the Department of Energy (DoE) last month claiming that the winning design from the Southeastern Universities Research Association (SURA) could not work, the effect was explosive.

Accelerator designers now agree that solutions to save the design are readily available; still, the incident has revealed just how political the matter has become, and it may also have raised questions about the expertise of the physicists who drew up the design. The story began on 4 June, when Fred Mills of Fermilab wrote to James Leiss, head of DoE's high-energy and nuclear physics office, to say that in looking over the SURA design he had discovered what he described last week as "a rather serious error". The problem was that the designers had based their assumptions of the performance of the electrostatic septa — used to extract the electron beam from the accelerator ring — on performance in existing proton accelerators. Mills pointed out that in an electron machine, the wires in the septa are a cathode, and field emission of electrons occurs — not a problem in proton accelerators where the charge is of the opposite sign. The difference could mean that field strengths of only one-half the design value can be achieved in the electrostatic septa.

DoE officials and SURA scientists wasted no time in trying to dispose of the objection. And DoE officials and at least some members of the Nuclear Science Advisory Committee (NSAC), which endorsed the SURA design in April, remain extremely touchy about the subject. Leiss said he understood the matter to be a "private" one between himself and Mills and refused to provide copies of any of the correspondence. He insisted that the NSAC panel that evaluated the design proposals had already raised the matter with SURA and that the required modifications would if anything decrease the costs of the project.

Similarly, Alan Bromley of Yale University, who chaired the NSAC panel, labelled it a "non-issue" and "trivial", and suggested that Mills's comments may have been "shaded" by his having designed an alternative approach to beam extraction. Bromley also made light of the notion that the SURA designers could have overlooked

the effect of the changed polarity in the septa. "Any damned idiot", he said, knows the difference between positive and negative charges.

SURA scientists have been much more open about discussing the problem. James McCarthy of the University of Virginia said "there are 20 alternatives; we've chosen the simplest solution, which is to modify the magnetic septa downstream". By altering the position and strength of these components, they can live with the halved field strength of the electrostatic septa, he said. McCarthy, too, asserted that the problem had already been considered by SURA and the NSAC panel.

Yet no mention of the problem appears in the panel's report. And although the technical sub-panel was apparently concerned about attaining the design field strength, it is not clear whether it specifically addressed the problems arising from the reversed polarity.

Hermann Grunder of Lawrence Berkeley Laboratory, who chaired the technical sub-panel, admits that the SURA designers did indeed overlook the consequences of the reversed polarity, and that it should have been mentioned in the report. But, he said, it does not alter the overall conclusion that "SURA had the better and more attractive design and environment".

And Grunder stressed that it just did not make sense to single out the polarity problem as an isolated issue. Grunder said he is more concerned about other problems, mentioned in the report, such as the work on the klystron tubes. He added that the political infighting was merely increasing the chances that DoE will take the easy way out and "the whole electron facility may get canned".

The choice between SURA and Argonne is now in the hands of Energy Secretary Donald Hodel. Hodel last month met Senator Charles Percy (Republican, Illinois) and a delegation from his home state who came to press the case for Argonne. Hodel will hear the other side next week from the Virginia congressional delegation. If construction is to begin in fiscal year 1985, as planned, Hodel would probably need to make a decision shortly so that research and development funds could be allocated for fiscal year 1984, which begins on 1 October this year.

The SURA board's recent vote to keep Newport News, Virginia, as the proposed site for its accelerator, may — however marginally — tip the scales in Argonne's favour. The NSAC panel had suggested that SURA reconsider this decision, noting the remoteness of that location from a university campus or an international airport. SURA is apparently banking on the decreased costs that the Newport News site offers because of existing buildings that can be used.

Stephen Budiansky

Malaria vaccine

Conflicting interests at work

Canberra

DELICATE negotiations are under way to settle how best to try to develop a malaria vaccine on the basis of the successful cloning of some of the parasite's genes by scientists at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia (see *News and Views*, p.13). The problem is how to satisfy the demands of the biotechnology companies that will be involved while protecting the interests of those organizations that have supported the research, particularly the World Health Organization (WHO). The same problem confounded initial attempts to involve biotechnology companies in the development of another malaria vaccine based on research at New York University Medical Center (see *Nature* 7 April, p.473).

The Hall Institute's malaria research project is funded by grants from WHO, the National Health and Medical Research Council and from the Rockefeller Foundation's great neglected diseases programme. The small but significant contribution from WHO, which has contractual requirements for public access, may deter domestic biotechnology companies from bidding for development rights. Nevertheless, Dr Mitchell feels that WHO requirements are "not obstructive" and some negotiation is possible. The situation calls for ingenuity in drawing up contracts so as to safeguard the interests of all parties. A meeting next week between the scientists and industry — the Commonwealth Serum Laboratories and Biotechnology Australia — will work out details of a formula for development. The Hall Institute is particularly anxious to safeguard the interests of Papua New Guinea in assuring its access to vaccine as serum and parasite samples for the project were supplied by the Papua New Guinea Institute of Medical Research.

When announcing the success earlier this month, the director of the institute, Professor Sir Gustav Nossal, said that the development of the vaccine within Australia was a venture tailor-made for support by the recently revived National Biotechnology Program (see *Nature* 23 June, p.648). However, if the government decides to support the companies involved or guarantees to buy the vaccine, the costs are likely to be outside the budget of the National Biotechnology Program. The Department of Science and Technology may be loath to support a product not regarded as a commercial proposition. Whatever the outcome of the meeting next week, the Department of Science and Technology will look at all funding options, including referring the problem to the Department of Health. **Vimala Sarma**

Fluoridation

Reluctant ban in Scotland

BOTH sides in the battle over fluoridation of domestic water supplies were claiming partial victory last week after the first test case on the matter in the United Kingdom.

Lord Jauncey ruled in the Edinburgh Court of Session that it was beyond the powers of Strathclyde Regional Council to fluoridate water supplies, as it had planned to do, but accepted that one part per million of fluoride in water reduced dental decay and had no significant adverse effect on health. Mr P. Clavell Blount of the National Anti-Fluoridation Campaign said he was delighted that Lord Jauncey had ruled against fluoridation; the council, on the other hand, was "pleased" that fluoridation had been vindicated as harmless.

The petition to prevent the council from fluoridating water supplies was brought by Mrs Catherine McColl, a 69-year-old pensioner from Glasgow, who describes fluoride as "a horrible poison". The hearing, which started in September 1980, was the longest in Scottish legal history and lasted for 204 court days. The council brought an impressive range of expert witnesses to argue for the virtues of fluoridation and to refute the alleged link with cancer. Mrs McColl, who was conspicuous by her absence after the first day of the hearing, relied on US anti-fluoridationists Dr Dean Burk and Dr John Yiamouyannis.

The 120,000-word judgement hinged on the interpretation of the Scottish Water Acts of 1946 and 1980, which enjoined the water authorities to prove "wholesome water". Lord Jauncey accepted that it was unlikely that parliament had intended in

1946 that water supplies should serve as convenient means of achieving a beneficial effect on health of consumers. Some consumers would not be of an age to benefit from fluoride (or — like Mrs McColl — have no teeth) and their freedom of choice would necessarily be restricted.

Lord Jauncey went out of his way to describe Dr Burke as "rambling and evasive", while Dr Yiamouyannis "allowed his hostility to obscure his scientific judgement". The evidence for excess cancer deaths linked to water fluoridation was, said the judge, "vague and unimpressive".

A spokesman for Strathclyde Regional Council said it was only a matter of time before fluoridation was introduced: "we may have lost the battle but we will win the war". The council is asking for talks with Mr George Younger, Secretary of State at the Scottish Office, seeking new legislation.

The more important implications of the case will first arise south of the Scottish border, where more than 10 per cent of the population already drinks artificially fluoridated water. English and Scottish law in this area are substantially similar, and there will now be pressure on local authorities in England and Wales to cease adding fluoride to water supplies. The Law Society says that a second test case for England and Wales is now on the cards, but the most serious question is whether the British government and parliament will have the stomach for new legislation if it should be needed.

Tim Beardsley

Margaret Thatcher, FRS by narrow vote

By a slender majority, the Fellows of the Royal Society decided last week that the Rt Hon. Mrs Margaret Thatcher "has done much to strengthen the cause of science in the United Kingdom" and so should be elected to join their ranks. Those angered by the citation failed by only a few votes to stop her election.

The election took place under a special statute of the society that allows its governing body, the council, to nominate persons who "have rendered either conspicuous service to the cause of science or are such that their election would be of signal benefit to the society". Opposition to Mrs Thatcher's fellowship centred on the view of some fellows that her service to the cause of science was conspicuous mainly by its absence and that ceremony should be cast aside in memory of shrinking university research and absent overseas students. But, when it came to the election last Thursday, just over the necessary two-thirds majority of the fellows present voted for Mrs Thatcher.

She joins Harold Macmillan and Sir

Harold Wilson in being elected FRS while Prime Minister. Wilson's election, while Lord Blackett was president of the Royal Society, caused a good deal of acrimony and opposition. There was a feeling thereafter that no further attempts to elect serving prime ministers should be made. Why the current council under its president, Sir Andrew Huxley, chose to risk nominating Mrs Thatcher is not clear. Having done so, the fellows are said to have been warned by Huxley that to reject her would be to do the society grave damage.

Last week's election meeting was said to have been much more sizeable and emotional than is usual for such occasions, the last of which was in 1976, when Sir Karl Popper was elected. Because there was no election of a fellow under the statute last year, a second election was allowed this year — David Attenborough, the film maker best known for *Life on Earth*. It is uncertain if the few votes cast against his election were from an anti-television lobby or from fellows who thought they were opposing Mrs Thatcher.

Peter Newmark

European research

Science council plans big

Brussels

SCIENCE ministers of the European Community last week agreed that Community spending on scientific research should be increased. But, with the coffers empty and a budgetary crisis building up, will the money materialize?

Speaking after the science ministers' council meeting in Luxembourg, chairman Heinz Riesenhuber, the West German federal research minister, announced "we've agreed on the need to increase funding, but how this is to be done goes beyond our competence". Present spending on research and development accounts for around 2 per cent of the European Community budget of 25 million ECU (one European Currency Unit = £0.58). Research commissioner Etienne Davignon is pushing for a doubling of research spending to 4 per cent of the budget by next year.

Other achievements of the meeting, Riesenhuber explained, were the endorsement of a Commission proposal for a four-year "framework programme" for research and development, and enthusiastic approval for Esprit, the programme for research in information technology. But "financial strains" made it impossible to give definite commitments on the asked-for 3,750 million ECU for the framework programme or the 750 million ECU for Esprit, the German minister said.

The monetary crisis is to be tackled in Athens on 5 December at the next summit meeting of European Community leaders. They will be looking for a way of curbing excessive farm spending and at the same time increasing support for other projects. Since this brings into question the structure of the controversial Common Agricultural Policy, and since some countries do not agree that the overall community budget should be increased, the summit could well end in deadlock.

The science ministers meet soon after the December summit, and no decisions to begin new projects or increase spending on existing ones can be expected before the hoped-for budget restructuring. And in this light, says a Commission official, "the Davignon line of gathering seemingly anodyne undertakings from member states to support scientific programmes without specific financial commitments could prove to be a very clever move".

If a new budget is agreed at the December summit, finance ministers will have just three weeks to adopt the 1984 financial scheme. And, says the Commission official, "if they skip research and development programmes, or sharply reduce asked-for financing, they could be in big trouble with a European Parliament entering its electoral period".

Geert Linnebank

US industry-university deals

Monsanto link lasts well

St Louis

ONE year after the announcement of a \$23.5 million five-year sponsorship of biological research at Washington University (WU), St Louis, by the Monsanto Company, both partners seem reasonably happy with the arrangement. The outstanding worry at the university is that Monsanto's cash may give other granting bodies an excuse to be mean.

When the scheme was first announced, there was predictable concern. Would scientists be limited in their freedom to publish? Would the direction of their research be changed to fit into the aims of industry? Would other sources of money be jeopardized? And how well would an internal peer review system cope with the distribution of such large sums of money — \$6 million in the first year and ultimately \$8.5 million on basic research plus \$15 million on "speciality projects" related to proteins and peptides that regulate cells?

The scientists who accept Monsanto money are assured that they will be free to publish, but Monsanto reserves the right to review manuscripts before they are submitted to check for patentable material. The university will hold any patents, but Monsanto will have the right to license any of them. No royalties will go to an individual researcher; instead, the money will go to the university and to the researcher's department and laboratory. Monsanto has 30 days to review a manuscript, and it can delay publication long enough to start the patent process.

Manuscripts are already going through the review process, and patent negotiations are under way for at least one finding. The review process so far has been smooth. One researcher says he submits a first draft of a paper to be published when he is about a month away from submitting it, so he does not feel that publication is delayed.

But publication will be awkward for WU researchers in another way — it will identify them as recipients of Monsanto money. Could this harm their chances of getting or keeping federal support? Many at the university are afraid that their colleagues on National Institutes of Health (NIH) review committees will feel that they no longer need NIH support because of the availability of the money from Monsanto. The fear is "an elusive thing that we think is real," according to Edward L. MacCordy, WU's associate vice-chancellor for research. The amount of NIH money coming to WU in the past year was up slightly compared with the previous year, but any effect of the Monsanto programme on NIH funding would not be expected to show up for a few years.

Other sources of funds that may be available to an applicant are supposed not to affect a peer review, but at a time when money is tight, any bias that might affect

the priority rating a researcher may get can be serious. Such bias has indeed surfaced overtly on one occasion, according to Dr David H. Kipnis, head of the internal medicine department at WU and the Monsanto project director for the university. During a site visit by an NIH review team, the head of the team held up a news article on the Monsanto agreement and asked if any money from Monsanto was involved in the project under review. Dr Kipnis protested to NIH, but no reprimand was given to the team's captain, he says. Peer reviews should focus on the quality and relevance of the research involved — not on the relative affluence of the institution, Dr Kipnis argues.

Grant applications under the Monsanto programme are reviewed by a committee of four scientists from WU and four from Monsanto. Faculty members were asked in November to submit letters of intent to file applications. Dr Kipnis says 17 or 18 letters were received, and seven or eight of those projects were supported. One more may still be approved for this year.

The WU and Monsanto committee members agreed quite closely in choosing the projects to be funded, and each of the eight on the committee gave top priority to the same eight projects. An outside review will be conducted at the end of three years to determine whether the agreement is to be renewed for another five years.

The agreement has not so far affected the direction of research at the medical school, but that may only be because most of the projects funded by Monsanto were already under way when the new programme started. For research of interest to Monsanto that is already being done at WU, the agreement offers a way for researchers to be sure that anything they find that can benefit human health will do so if it suits Monsanto.

The head of one laboratory, who wished to remain anonymous because of the problem with NIH support, said that the money from Monsanto had enabled him to support junior faculty and to buy equipment, but the company has resisted the temptation of trying to interfere in the running of his laboratory. "I do not feel pushed or directed or influenced in what I do," he said. He will be using Monsanto money to make up for a reduction in NIH support. He lost the money for a technician when his last NIH grant was renewed, a cutback which was part of the general belt-tightening at NIH. Part of his Monsanto money will go towards keeping that technician working.

While WU's scientists hope to benefit from the programme without compromising their academic integrity and freedom, Monsanto, of course, hopes to make money. The company is optimistic enough about research into factors that affect

blood clotting, regulation of the immune system and wound healing to have formed a Health Care Division in January this year.

Dr Howard A. Schneiderman, senior vice-president for research and development at Monsanto and the moving force behind the agreement with WU, says that the company is actively fostering a close relationship between its scientists and those at the medical school because this sort of cooperation is "almost necessary" for US corporations to compete in world markets. Other countries allow companies to pool their research resources in consortia, but in the United States antitrust laws make cooperation with universities the most attractive option for private industry, Dr Schneiderman said (see, however, page 4). He contends that the cooperation benefits both parties: Monsanto can accelerate the pace of research at WU, and WU scientists can give Monsanto in-depth counsel on company projects.

Karen Freeman

Bristol explorers

PLANS for a new "interactive science centre" at Bristol seem to be advancing well. The committee responsible for the project has now appointed a full-time coordinator and a likely building has been found in Bristol's dockland.

The new centre, which is to be called the "Exploratory", is partly inspired by the San Francisco Exploratorium. Professor Richard Gregory of the University of Bristol, who initiated the idea, prefers not to use the word "museum" for the Exploratory, which will allow young people to repeat classic experiments in physics and learn about perception by witnessing visual illusions. The theme linking the exhibits will be "the individual as observer".

Initial costs have been met by the Nuffield Foundation. The project advisory board is now aiming to raise £1½–2 million to equip the Exploratory, which it is hoped will open in 1985. Approaches will be made to industrial companies with an interest in science and technology, and there are hopes that the Department of Industry may be persuaded to chip in.

The building is a 9-storey disused warehouse with 200,000 square feet of floor space. Only two or three floors will be used for the Exploratory; Gregory hopes to fill the others with local societies and small companies as a source of revenue. The site is being negotiated with Bristol City Council, which plans to develop the whole dockland area. A new railway link will allow easy access. Once established, the Exploratory will aim to become self-supporting, although Professor Gregory wants the admission fee to be sufficiently low that young people are not deterred. Advice and some exhibits have been given by the San Francisco Exploratorium.

Tim Beardsley

Facts, not rhetoric on yellow rain

SIR — R.D. Caldwell, in his letter "Yellow rain" or natural toxins?" (ref. 1), comments and expands on the feasibility of naturally occurring mycotoxins in South-East Asia and advocates the open exploration of alternative hypotheses instead of the trading of unfounded accusations.

It is unfortunate that Caldwell, in his eagerness to get his point across, makes use of unfounded arguments himself. He refers in his letter to Canadian investigations² in which, according to Caldwell, it is reported that trichothecene-producing fungi, such as *Fusarium semitectum* and *Fusarium sporotrichioides*, were found in most samples collected near the Thailand-Kampuchea border.

While the report² is not very specific as to the actual number and types of isolates (this work is still in progress and not yet published), nowhere in the report does it say that *F. sporotrichioides* was isolated. I presume that Caldwell misinterpreted the tentative identification (page 25 of the report) as the final diagnosis. In fact, the text on page 25 continues by stating explicitly that this suspected strain of *F. sporotrichioides* was finally classified as *F. semitectum*.

Also, I regret Caldwell's premature conclusion that the isolates were trichothecene-producing fungi. It is well known that the identification of a particular fungus does not give any clues as to the toxigenic potential. The toxigenic potential of the isolates of the Canadian study has not been determined yet, thus we do not know what potential they might have. However, one should recognize at this point the fact that the chemical analysis of the environmental samples from which the fungi were isolated did not reveal the presence of any mycotoxins. (Incidentally, *F. semitectum* was isolated only once from all these samples.) The only possible hint that toxigenic *Fusarium* spp. may exist in Thailand can be found in a recent publication³, in which it is stated that an extract of a culture of *F. semitectum* isolated from a "yellow rain" spot caused death of experimental animals, but the chemical analysis of the extract remains to be done. Another article in *Nature*⁴ misquotes this Thai paper by stating that "*F. tricinatum*, which produces the mycotoxin T-2" was found in 22 of the specimens of yellow rain. What the article actually says is that *F. semitectum* (not *F. tricinatum*) was not found in 25 sampling places on the leaves apart from the yellow rain spots.

Further, I am rather critical of Caldwell's statement that T-2 producing *Fusarium* spp. were described in the 1930s in Vietnam. At that time, nobody knew of T-2 toxin, and all that one can say is that *Fusarium* species, which might be able to produce T-2 toxin, were found in Vietnam in the 1930s, but their toxigenicity was

never assessed.

In view of the established facts, I find it premature to conclude, as Caldwell does, that trichothecenes occur naturally in South-East Asia. I find it even more premature to conclude that the events that are described as occurring in Kampuchea and Laos can be explained on the basis of natural occurrence of toxins.

I invite Caldwell and anyone who has the scientific capability and resources to conduct the much needed studies, that is, to investigate the matter of natural occurrence of mycotoxins, particularly trichothecenes, in South-East Asia. It is not rhetoric or scepticism, which leads to misinterpretation of data, that will help solve this problem; what we need are hard scientific facts.

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Vote for A?

SIR — R.M. May¹ raises again the difficulties facing a system of voting to fill a single place, and in particular the paradox (A beats B, B beats C, C beats A) that can arise if Condorcet's system of pairwise comparisons is used. He writes, however, that his general impression is that such paradoxical results "are not likely to be too common in practice".

I should like to comment, as someone who has used Condorcet voting in practice, mainly within the council of the Royal Statistical Society. In more than a dozen uses I have never met a circularity for the first place yet. Of course, such a result, or a tie, could occur and rules must be drawn up for what happens next if so, but this is no worse than the fact that a tie could occur in any voting system.

Where election is to fill a single position, such as a president, a pope or a party leader, it has enormous advantages over any other system, namely: (1) It completely eliminates the effect of "split" votes and tactical voting (unless the paradox intrudes). (2) It is therefore very easy for the voter to use, as the only sensible ordering of the candidates is by *true* preference. (3) Anyone can stand for election without worrying about spoiling someone else's chances relative to any other candidate. (4) Unless the paradox arises it gets the right result (that is, the candidate who could have won a straight fight against any one of the others) and does so in only one round of voting. (5) If the paradox arises it still does not give the wrong result but is merely indeterminate.

The difficulty lies not in the system itself but in convincing people that it is the right system to adopt. The situation is not helped by the publication of a recent book by R.A. Newland² which includes a chapter on "Filling a single place". This chapter correctly shows that no system is really worth consideration except Condorcet or the "alternative vote" method, but then says "the alternative vote is the preferred method", without mentioning any of Condorcet's advantages. As the author is a past chairman of the Electoral Reform Society, his advice may unfortunately be taken seriously.

It should be noted that Condorcet is not a contender for electoral reform where proportionality is required. It is no more a proportional system than is our present parliamentary electoral system. It can be used, however, to elect more than one candidate where proportionality is not required. For example, it would be ideal for electing, or re-electing, four teams each year to the Fourth Division of the Football League.

It is sometimes contended that the counting is difficult. In practice, it is much easier than might be thought at first glance, but in any case that should not really be a consideration nowadays when a computing algorithm³ can be written to do the task.

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Foothills and corridors

SIR — Concerning Sydney Brenner's pessimistic vision of molecular biology (*Nature* 21 April, p.654), may I offer a countervision — that on cloudy or hazy days we can but see "the foothills" despite the nearby ranges; and that the successive "long corridors" which are molecular biology today, and which have doors only at the distal ends, may also have high windows, or cracks in the walls, such that only those tall enough or sufficiently astute can see out. Ultimate liberation might be the privilege of those who attempt to break away from the constraints of the corridor by enlarging the cracks or by going through the window (vandalism, of course). Isn't this exactly a "paradigm-shift", the concept you assail (*Nature* 14 April, p.557)? In this context what was ascribed to Brenner should perhaps read: "[the long-corridors story] is what *normal* science is like". Understandably, what one can do in Kuhnian normal science is "just [to] publish more and more. . .".

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The temptations of numerology

Too much innocent energy is being spent on the search for numerical coincidences with physical quantities. Would that this Pythagorean energy were spent more profitably.

EVER since the Pythagoreans sought an explanation of their puzzling world in terms of simple numerical relationships, there has been a small but ingenious industry given over to the search for numerical relationships between supposedly fundamental quantities that specify the Universe. And the numerologists do have a few successes to their credit. Prout's hypothesis, early in the nineteenth century, that the masses of atoms relative to hydrogen would turn out to be integers when accurate measurements were carried out was for a long time discredited when accurate measurements of chlorine, for example, seemed to make such a simple rule untenable. But as things have turned out, atomic masses are indeed multiples of the nucleon mass with an appropriate allowance for what was called the "packing fraction" half a century ago. Much the same may be true of what is known as Bode's law, the rule that the distance in astronomical units from the Sun to the Solar System planets is given by a simple arithmetical series, at least as far as Neptune and if it is supposed that the asteroid belt takes the place of the planet between Mars and Jupiter supposed to be "missing". For although none of the attempts so far to account for Bode's law by the condensation of material from the solar nebula is wholly convincing, there is always a chance something may turn up.

These are not, however, the fields in which the numerology industry concentrates its efforts. With the proliferation of different particles of matter in the past several years, most of the practitioners have turned their attention to the discovery of numerical relationships between particle masses. Some have been impelled in that direction by the recognition that the reciprocal of the fine structure constant, a dimensionless quantity, is almost (but, significantly, not quite) the integer 137. More than a score of papers in this genre turn up in the *Nature* office each year, and then make their way back to their authors. The accompanying argument, from Mr Peter Stanbury, a factory worker from Tunbridge Wells in Kent, is a comparatively elegant piece of numerology which the author fears may be kept from the public by a stuffy establishment.

There are three kinds of reasons why numerology usually gets and more often deserves scant attention. First, if indeed there is some underlying numerical ideal, why are the relationships never exact? How

can Pythagoreans be so tolerant of departures from their golden rules, in this case by several parts in a thousand? Second, sheer coincidence is by no means as unlikely as the numerologists like to think. Indeed, given that simple algebraic combinations of numbers such as π are literally infinite, the chance of being able to match an arbitrary set of numbers to within a fraction

of a per cent must be high, tedious though the task may be. Third, there is the sceptical riposte "So what?". In spite of the hard work lavished on these numerical comparisons, nobody is any wiser about the way that matter is constituted even when they are successful. The pity is that such devotion is spent on such fruitless pursuits.

John Maddox

The alleged ubiquity of π

IT has long been known that the proton to electron mass ratio is very nearly $6\pi^5$ —that is to say that $m_p/m_e = 1,836.15152$ compared with $6\pi^5 = 1,836.118$. What I have done is to look for and find a more general relationship between the value of π and the masses of the sub-atomic particles.

The particles of the basic octet are π^0 , π^+ , π^- , κ^+ , κ^- , ϵ^0 , Ξ^0 and η . The sum of their masses is $3.14006 m_p$. It can hardly escape one's attention that the multiplier of m_p is very nearly π or 3.1415926 . When I first discovered this, my natural reaction was to see if anything similar occurred for baryon masses. The basic baryon octet contains the particles p, n, Λ , ϵ^+ , ϵ^- , ϵ^0 , Ξ^0 , Ξ^- and the sum of their masses is $9.812 m_p$, where the multiplier is significantly close to π^2 or 9.869604 . Can one really say that both these results are coincidence?

Having observed that $m_p/m_e = 6\pi^5$, let us do away with man-made electron volt units for mass and instead use a system in which $m_p = \pi^6$ so that $m_e = \pi/6$. In these units, the masses of the particles become

$$m_p = 961.389 (= \pi^6)$$

$$m_\mu = 108.26$$

$$m_{\pi^0} = 138.28$$

$$m_{\pi^\pm} = 143.006$$

$$m_{\kappa^\pm} = 505.827$$

$$m_\eta = 562.3$$

$$m_\epsilon = 0.5235987 (\pi/6)$$

I note that

$$m_\mu/\pi^4 = 1.111434 \quad (1)$$

$$m_n/m_{\kappa^\pm} = 1.11167 \quad (2)$$

$$(m_\eta + m_{\kappa^\pm})/m_p = 1.1102 \quad (3)$$

Relations (2) and (3) are both independent of the choice of units. Note also that $1 + \pi^{-2} + \pi^{-4} = 1.111587$.

Now we come to the really interesting part of my work. The fine-structure constant is a dimensionless quantity whose reciprocal is equal to 137.03604 and is very nearly equal to $4\pi^3 + \pi^2 + \pi$ or 137.03630 . Considering that this represents the ratio of the strength of the strong nuclear force (for which π^0 is the main carrier) to the electromagnetic force, it must surely be of some significance that the value of m_{π^0} is $\pi^4 + \pi^3 + \pi^2$ or 138.286 .

How does all this fit together? For a long time I could see no really simplified pattern until I found the following:

	x	y	z
A	π^4	π^2	1
B	π^4	π^3	π^2
C	π^4	π^4	π^4

The values in row B (column y) add up to the value of m_{π^0} already quoted. The values in row A (column z) add up to 108.276 , which is close to the value 108.2618 for m_{π^0} . The values in row C (column x) do not add up to a particle mass, but the sum of rows B + C (columns x + y) is the same as the expression I have previously derived for $\pi\alpha^{-1}$. Although row C does not produce a mass value, $m_\mu + m_{\pi^0} + m_{\pi^\pm} = 4\pi^4$.

Bearing this in mind, we note that

$$m_{\pi^\pm}/m_{\pi^0} = 1.03441 \quad (A)$$

$$(m_{\pi^\pm} - \pi^3)/m_\mu = 1.0345 \quad (B)$$

$$m_{\pi^0}/(m_{\pi^\pm} - \pi^3) = 1.1111^2 \quad (C)$$

The two values 1.0345 and 1.1115 turn up in so many of our results that I would state categorically that coincidence is ruled out. Indeed, there are many more of them, of which the following are two examples

$$\pi^{11}/m_\eta \cdot m_{\kappa^\pm} = 1.0343$$

$$[(m_\mu/m_\eta) - m_{\pi^3}]/m_{\pi^\pm} = 1.0346$$

Peter Stanbury

Cancer

Oncogenes and growth factors

from Robin Weiss

THE product of an oncogene has for the first time been identified as a protein with a known physiological effect in normal cells. Two laboratories have independently discovered that the *sis* oncogene encodes a protein similar or identical to platelet-derived growth factor (PDGF). In this issue of *Nature*¹, M. Waterfield and associates show that a region of 90 contiguous amino acid residues of PDGF is virtually identical to the recently published predicted sequence² of a large domain in p²⁸*sis*, the putative transforming protein of simian sarcoma virus. Doolittle *et al.*³ have discovered the same correlation, based on somewhat less extensive PDGF sequencing. Both discoveries arose from a computer search of the NEWAT protein sequence data base established at the University of California in San Diego by Doolittle⁴. Antoniadis and Hunkapiller⁵ had recently published data on partial PDGF sequences and had not noted any homology with sequences for other growth factors. When Doolittle entered these sequences into his data base and searched for sequence homology, the striking similarity to p²⁸*sis* was revealed. Waterfield *et al.*, using the same data base by courtesy of Doolittle, discovered the same homology before publishing their PDGF sequence. As the cellular homologue (c-*sis*) of the viral *sis* gene is present in the human genome as a unique gene⁶, in all probability it is the gene for PDGF.

It has been difficult to establish contiguous sequences of PDGF because the purified factor contains several cleaved peptides linked by disulphide bonds at cysteine residues. In this respect it resembles epidermal growth factor (EGF). However, by cDNA cloning the EGF mRNA, it has recently been shown that EGF is derived

from a much larger precursor polypeptide⁷ which may contain other active peptides, as found for many peptide hormones. If c-*sis* is indeed the gene for PDGF, then the ordering of the PDGF peptides and the identification of precursor molecules becomes much easier, as Waterfield *et al.* discuss in their paper¹.

PDGF is released from α -granules of platelets during blood clotting and it is the major polypeptide growth factor found in serum. Fibroblastic and neuroglial cells are specially sensitive to the mitogenic action of PDGF, as they express PDGF receptors⁸. Like the EGF receptor⁹, the PDGF receptor exhibits tyrosine-specific kinase activity on binding the growth factor¹⁰. This type of protein phosphorylation is reminiscent of the enzyme activity of the *src* family of oncogenes¹¹. Thus it appears that oncogenes such as *src* and *abl* encode a growth factor itself. Provided that the same cell produces both factor and receptor, then autocrine stimulation¹² of proliferation will ensue. It is noteworthy that those human tumours found to express the

cellular homologue of the *sis* gene are sarcomas and gliomas¹³, derived from cell types sensitive to PDGF.

Many cells transformed by oncogenic viruses release mitogenic 'sarcoma growth factors' resulting in autocrine growth stimulation^{14,15}. Activated human *ras* oncogenes in transfected NIH 3T3 cells also stimulate release of sarcoma growth factor¹⁶. In these cases, however, the oncogene does not appear to encode the growth factor, but secondarily to induce its synthesis or release from the transformed cells. Two transforming genes, however, have been shown to share at least a partial homology with mitogenic peptides. First, Baldwin¹⁷ has found homology between polyoma virus middle-T antigen and gastrin, which can act as a mitogen¹⁸. The homology is particularly strong around the tyrosine site that becomes phosphorylated¹⁹. Second, the *B-lym* gene of chicken bursal lymphomas, identified by DNA transfection of NIH 3T3 cells, encodes a small polypeptide that shows partial homology to the amino terminus of transferrin²⁰. The apparent identity of *sis* with PDGF is nonetheless the first example of an oncogene representing a previously defined growth factor. □

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Astronomy

Half a century of radio astronomy

from J.S. Hey

FIFTY years ago, on 8 July 1933, a letter of Karl Guthe Jansky of the Bell Telephone Laboratories, USA, entitled 'Radio waves from outside the Solar System' was published in *Nature*¹. There he announced that his research on radio atmospherics had revealed extraterrestrial radio emission from a region in the vicinity of the centre of the Galaxy. Jansky had first described his discovery at a meeting of URSI in Washington on 4 May 1933. The news was considered sufficiently startling to warrant a full-column report on the front page of the *New York Times* of 5 May.

Despite this promising beginning interest lapsed for more than a decade except for the extraordinary enterprise of an American radio engineer Grote Reber who in his spare time erected in his back garden a steerable radio telescope of 9.5-m diameter. With this equipment he traced the first sky map of the radio source, clearly demonstrating its close correspondence with the Milky Way.

Several factors contributed to the slow rate of advance of research. Astronomy and radio occupied separate domains of scientific activity. Further, the long radio wavelengths seemed to present an insurmountable obstacle to the possibility of attaining angular resolution comparable to

that of optical images.

Discoveries during and following World War II triggered the development of modern radio astronomy. In 1942, I was responsible at the Army Operational Research Group (AORG) for investigating jamming of British army radar, and reported intense radio bursts from the Sun. At the time it was feared that anti-aircraft radar might be rendered ineffective by airborne jamming. On 27 and 28 February 1942 radars operating at a few metres' wavelength experienced severe noise jamming. Alarm was widespread but fortunately no air raids were in progress. The daytime occurrence and directional measurements of the noise indicated powerful radio emission from the Sun. On telephoning the Royal Greenwich Observatory I was informed of the presence on the Sun of a large and exceptionally active sunspot, and it seemed natural to attribute the abnormal radio emission to this region. The event was so unexpected that the conclusion was received with scepticism by several radio scientists. However, the director of AORG, B.F.J. Schonland, recalled Jansky's discovery of galactic radio noise, and my report was generally accepted.

Later events during the war again led me

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to the investigation of radio astronomical phenomena. In 1943 when the danger of the V2 long-range rocket attack was imminent I proposed the use of modified army radar for the warning and tracking of approaching rockets. The scheme proved successful, with a chain of watching radar installed by AA Command around the south-east coast. Subsequently the system was developed to predict the point of fall. (In fact, AA Command planned to fire a dense barrage to intercept the rockets and explode them in mid air but the V2 attack ceased before this defence could be tested.)

The operation of this rapidly improvised system brought two unsuspected problems. Spasmodic transient echoes might be attributable to meteoric ionization, but there was no certain proof. Also, attempts to improve radar sensitivity by the installation of low-noise receivers produced no improvement. A suggestion that the sensitivity was actually limited by the galactic radio noise that Jansky had discovered was soon confirmed by checking the directional variation of the radio noise. With the end of the war in 1945 my team pursued its investigation of the phenomena. We demonstrated indisputably that the 'short-scatter' echoes were produced by meteor trails and we derived meteor radiants and velocities; and detailed mapping of the radio noise from the Galaxy led to the first detection of a distant discrete source of radio noise (Cygnus A). When security restrictions were eventually lifted an account was published in *Nature*². Further letters to *Nature* described the first radar studies of meteor trails³, and the detection of a localized source of radio emission within the constellation of Cygnus⁴.

These discoveries led to the inauguration of three major radio astronomy research groups, led by M. Ryle at Cambridge, A.C.B. Lovell at Manchester (Jodrell Bank) and J.L. Pawsey at CSIRO, Australia. Ryle and Pawsey began by studying solar radio noise, and they devised interferometers to locate the sunspot regions of enhanced emission. Lovell's original aim was to detect radio echoes from cosmic ray showers, for Blackett and Lovell had published a paper in 1941 suggesting that cosmic rays might be detected by radio echoes from the ionization they produce in the atmosphere⁵. Lovell decided that the radars of the V2 watch might be suitable for this purpose, and they and Parsons helped to install radar equipment at Manchester University. It soon became evident that a new site away from city electrical interference would have to be chosen. It also seemed certain that observed radar echoes would be predominantly those from meteor trails. Lovell was already planning a large radio telescope for the detection of cosmic-ray showers at a new site, Jodrell Bank. However, the project to observe radar echoes from cosmic-ray showers proved unfruitful and Lovell turned instead to radar studies of meteors. Following our announcement of the discovery of

the discrete radio source in Cygnus, the search for extraterrestrial radio sources became a main focus of interest of all the research groups.

With the aid of radio interferometers, other discrete radio sources were soon discovered⁶, and the improved angular resolution enabled Graham Smith at Cambridge⁷ and others to define the source positions more precisely. At Jodrell Bank where the potentialities of large reflectors were being exploited, Hanbury Brown and Hazard⁸ scanned the Andromeda Nebula and thus plotted the first radio map of an external galaxy. Radio methods quickly branched out to make important contributions to almost all branches of astronomy.

The outstanding successes of worldwide radio (and radar) astronomy research provide an epic saga of scientific progress. The catalogue of achievements is so overwhelming they cannot be summarized briefly. A specially prepared revised edition of *The Radio Universe*⁹ is being published to survey the principal accomplishments of the half century.

Malaria

Cloning genes for antigens of *Plasmodium falciparum*

from F.E.G. Cox

THE possibility of producing a vaccine against malaria is probably the greatest challenge that parasitologists have ever faced. The main problem is that each stage in the complex life cycle of the malaria parasite has its own unique antigens. The infective stage is the sporozoite which, having been injected directly into a capillary by a mosquito, rapidly enters the liver to undergo a massive phase of exoerythrocytic division, during which it neither elicits nor succumbs to any immune response. After about a week, minute merozoites are released from the liver and either invade red blood cells to undergo another cycle of division, or differentiate to become gametocytes that subsequently infect mosquitoes. The four major stages, sporozoite, exoerythrocytic forms, blood forms and gametocytes all have quite different antigens. Earlier and now clearly crude methods of isolating and identifying antigens have been superseded by the techniques of gene cloning. The first stage to be successfully investigated was the sporozoite which expresses one major protective surface antigen¹. Now David Kemp and his colleagues in Australia have extended the technique to the much more complex proliferating blood stages of the parasite².

In *Plasmodium knowlesi* from monkeys, the sporozoite antigen has a molecular weight of 42,000 and the precursor molecule, which is intracellular, one of 52,000 (ref.3). Cochrane *et al.*¹ used a monoclonal antibody against the surface

We now witness a new phase in astronomical research. X-ray astronomy has advanced rapidly, rivalling the postwar development of radio astronomy. Aided by observatories on space satellites the whole electromagnetic spectrum can be brought into view. It is evident that in addition to optical images and spectroscopy where greatly improved techniques have been devised, data in other wavebands ranging from γ rays, X rays, ultraviolet, infrared and radio are proving essential for a comprehensive understanding of astrophysical phenomena. □

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molecule as a probe to identify polypeptides produced by *Escherichia coli* into which *P. knowlesi* cDNA had been inserted. The experiments showed that a single mRNA encodes both the intracellular precursor and the protective surface antigen and confirmed that there is no cross-reaction between sporozoite and blood-stage antigens. In the case of the sporozoite, only one major antigen is involved in protection, and it has proved relatively easy to identify the antigen and clone the gene encoding it. The sporozoite is however only one stage in the parasite's life cycle and it is relatively fleeting in the mammalian host.

The blood stages are inherently more difficult to work with because of the numerous antigens that have already been identified, a lack of information about which of these is protective and difficulties in determining how this protection could be made effective. The human parasite *P. falciparum* can easily be maintained in culture⁴; these cultures can provide considerable amounts of antigen and make it possible to test the protective effects of immune sera⁵. This technical background has now allowed Kemp and his colleagues to isolate and clone several antigens from the blood stages of *P. falciparum* and to show that these are recognized by the sera from these are recognized by the sera from naturally recovered malaria victims². Using standard continuous-culture techniques, parasites were disrupted and their

mRNA extracted. This was then copied into cDNA and inserted into *E. coli*. The gene products were identified using immune sera known to inhibit the growth of *P. falciparum* *in vitro*. As a result of their work the Australian scientists now have a library of several hundred positive clones most of which are distinct and result from a variety of mRNAs. They conclude that the antigen-positive clones represent a significant fraction of the immunogenic proteins of *P. falciparum*.

This particular study is a significant breakthrough because it shows that the antigens of the blood stages of a human malaria parasite can be cloned and that these are recognized by immune sera. It will however be a long time before this can lead to an effective vaccine. The culture technique used for the production of parasites provides a heterogeneous collection of different stages; it is now quite clear that at least one of the most important antigens of *P. falciparum* is synthesized and expressed late in the developmental cycle at the time the merozoite begins to differentiate⁶, and it is not known whether merozoite antigens are among those encoded by the genes cloned by the Australian group. It will soon be necessary to clone the antigens as they are produced in sequence in a synchronous culture, to clarify this sort of question. Moreover there is a possibility that some of the antigens may turn out to be inserted in the infected cell rather than the parasite. And even when these questions have been resolved it will be necessary to investigate parasites from different geographical locations, collected at different times, because the antigens are known to vary over both space and time. But the most important problem is that, in contrast to the sporozoite antigen, the antigens that elicit protective responses to blood-stage parasites, and the stages at which they are expressed, are not known. There is abundant evidence that merozoite antigens elicit protective antibodies and some of the antigens detected in the Australian study² may actually be these.

Taken together, the two successes in cloning malarial antigens, from sporozoites and blood stages, take our understanding of the mechanisms of immunity to malaria and the possibility of a vaccine a step closer. It now remains for the antigens of the merozoites and gametocytes to be cloned. When this has been done it may be possible to take an empirical approach to vaccination based on a cocktail of antigens and possibly to bypass the need to understand the mechanisms involved in immunity. □

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DNA repair

Error-prone enigma

from Bryn Bridges

ULTRAVIOLET light, ionizing radiation and many mutagenic chemicals give rise to mutations in the bacterium *Escherichia coli* and in many of its phages through the action of an error-prone DNA repair pathway. This pathway, known as 'SOS' and the expression of which is controlled by the LexA repressor, is now fairly well defined (see *Nature, News and Views* **296**, 606 and **298**, 118; 1982). What is less well understood is the precise way in which the mutations arise. This was one of the major topics discussed at a recent international meeting*.

A favourite approach for dissecting bacterial mutagenesis has been the use of bacteriophage since the induction of the SOS system in the bacterium can be divorced from the induction of DNA damage in the phage. This makes it possible to distinguish mutations that do not depend on damage in the phage DNA (untargeted mutations) from those that do. The damage-dependent mutations that occur at the sites of damage are known as targeted mutations. Targeting and non-targeting of mutations was considered in detail at the conference.

Although it is inducible, untargeted mutator activity does not involve the LexA repressor (Ichikawa-Ryo and Kondo *J. molec. Biol.* **97**, 77; 1975), nor does it require the *umuC* gene (Hutchinson, Yale University; Caillet-Fouquet, University of Brussels). It is also absent in *recA* bacteria (*RecA* protein is normally responsible for cleaving LexA protein during SOS induction), suggesting it might represent a new inducible SOS function using an unknown repressor distinct from LexA.

In a carefully conducted series of experiments, Hutchinson has analysed targeted and untargeted mutations induced in the *cI* gene of λ phage by irradiation with 254-nm UV light. Most of the targeted mutations were base pair substitutions which were located between base-pairs 1 and 240 (specifying the amino-terminal end of the polypeptide). Two-thirds of them were transitions and 60 out of 62 of these were at sites with adjacent pyrimidines. Such transitions might therefore have arisen either from cyclobutane pyrimidine dimers or from the '6-4' dipyrimidine photoproduct described by Haseltine (Boston University). When the phages were irradiated with 313-nm light in the presence of acetophenone, a treatment which produces the former but not the latter lesions, very few transitions were found. This provides strong evidence that, at least in this system, the transitions after 254-nm UV arose not

at the sites of pyrimidine dimers but at 6-4 pyrimidine photoproducts postulated by Haseltine to be the principal mutagenic UV photoproducts. Since most transitions were at the 3' pyrimidine of the presumed photoproducts, Hutchinson proposed that the 5' pyrimidine is still able to base-pair and that only the 3' pyrimidine acts as a non-coding base. In contrast to the targeted mutations, untargeted mutations were predominantly frameshifts and were not confined to any particular region of the gene.

Lawrence (Rochester University) described an elegant mating system in yeast in which he has estimated that between 13 and 38 per cent of UV-induced mutations are untargeted. The untargeted mutator activity seems to result from a loss of replication fidelity due to sequestering of 'fidelity factors' by DNA damage at other sites rather than from induction of a diffusible error-prone enzyme or factor. Analogous experiments in *E. coli* using UV-irradiated incoming F' (single-stranded DNA) indicated a smaller proportion of untargeted mutations. Most of the targeted mutations were photoreversible, suggesting that, in contrast to the λ system, pyrimidine dimers rather than 6-4 photoproducts were the targets in this case.

Leclerc (Rochester University) used an M13 mp2 phage *lacI* forward mutation system in SOS-induced bacteria to study UV mutagenesis. He reported the results of sequence analysis of 117 mutants. 74 per cent of the base substitutions occurred at sites of adjacent pyrimidines, usually thymines, and there was a preference for mutations to occur at the 3'-pyrimidine site, as had been reported also by Hutchinson. There were many multiple non-tandem base-pair substitutions suggesting that an error-prone complex acting at a pyrimidine-pyrimidine photoproduct was also likely to make an error at a nearby undamaged site. Such mutational changes have been called hitch-hiking errors since, strictly speaking, they are neither targeted nor untargeted.

The first evidence for targeting in mammalian cells came from the work of Sarasin (CNRS, Villejuif) who sequenced revertants of a temperature-sensitive mutation in UV-irradiated simian virus 40 infecting UV-irradiated monkey cells. Although only 16 had been analysed, all were single base-substitution second-site mutations and all had occurred at two adjacent thymines.

Evidence is emerging that base insertion opposite a non-instructive template site is not random. Loeb (University of Washington, Seattle) reported that in SOS-induced cells, deoxyadenosine is the

*The UCLA symposium on 'Cellular responses to DNA damage' was held at Keystone, Colorado on 15-19 April 1983.

predominant insertion opposite apurinic sites in single-stranded Φ X174 DNA and Kunkel (University of North Carolina) obtained a similar result with heat-treated single-stranded M13 mp2 DNA. This is consistent with *in vitro* observations on DNA polymerase I by Loeb and by Strauss (University of Chicago). In terms of selective advantage during evolution this would seem sensible as the lesions most likely to be encountered by a DNA replication fork in significant numbers are sunlight-induced photoproducts involving adjacent thymines. Preferential insertion of deoxyadenosine in such a circumstance would result in conservation of the genetic information rather than mutation.

Although untargeted mutations were frequently found in infections of UV-irradiated bacteria by bacteriophage they were presumed to be less important in bacteria themselves because of the effectiveness of the bacterial mismatch-correction system. This system is able to

distinguish a newly replicated DNA strand from the parental strand by the former's paucity of methylated adenines and acts by correcting replication errors in the newly synthesized strand against the 'correct' sequence in the parental strand (Meselson, Harvard University). The same phenomenon has now been shown to operate in cell-free extracts of *E. coli* (Lu, Duke University). The *in vitro* activity is dependent on the state of the *dam* methylation of the DNA and is deficient in extracts of the mutator strains *mut-H*, *mut-L* and *mut-S*. The correction process involves a repair-synthesis event of considerable length (up to 1,000 nucleotides) which probably stretches between the mismatch site and the nearest *dam* methylation site. Both these sites (relatively remote from one another) have to be present for the repair synthesis to occur, which raises intriguing possibilities for the mechanism of action. □

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Celestial mechanics

Acceleration on Lageos spacecraft

from David E. Smith

SINCE the launch of the Lageos spacecraft in May 1976 into a near-circular orbit at 6,000 km altitude, the spacecraft orbit has been decaying at an average rate of about 1 mm per day. This decay causes the satellite to accelerate in its orbit at a rate of about $3 \times 10^{-12} \text{ m s}^{-2}$, which is one to two orders of magnitude larger than was expected. The cause of the acceleration (orbit decay) is unknown but calculations and theoretical studies of the changes in the spacecraft orbit suggest that charged-particle drag and Earth albedo radiation pressure (sunlight reflected from the Earth back onto the spacecraft) are the most likely explanations. Recently, the orbital decay seems to have ceased and even reversed, at least temporarily, so that the orbit may be beginning to increase again in size. If this is the case, charged particle drag cannot be the sole explanation and Earth albedo becomes a very probable second cause.

The Lageos spacecraft is a sphere 60 cm in diameter carrying 426 laser corner reflectors on its surface. The precision-built spacecraft was designed to be a near-perfect target for ground-based laser-tracking systems that could measure the spacecraft's distance (range) to better than a centimetre. The measurements were to be used to determine precisely the relative positions of the laser-tracking stations for estimating crustal movements, such as tectonic plate motion, and the rotation of the Earth on its axis.

Because of its design, high altitude and very accurate tracking, Lageos has the most precisely known orbit of any artificial satellite. Within weeks of its launch it became clear that the spacecraft was ac-

celerating in a manner consistent with drag (neutral or charged particles) but at a much higher rate than expected¹. Attempts to explain this fact² were initially directed towards the possible existence of helium at the satellite's altitude of 6,000 km but ran into the difficulty of requiring very large exospheric temperatures. Neutral hydrogen was also ruled out as the primary cause of the drag^{2,3} and attention became focused on the effect of charged particles. As the spacecraft moves through the atmosphere it becomes charged through collisions with charged particles and interacts electromagnetically with particles at a distance, thereby losing energy. Although we do not know the charge on the spacecraft it seemed that charged-particle drag could account for the observation³⁻⁵.

Several years of laser tracking of Lageos have shown systematic variations in the accelerations about the mean of around 100 per cent and with several different periods. The largest-amplitude period is about 285 days, half the time the orbit takes to precess in space with respect to the Sun. Although the variations in the acceleration are significant, none has been successfully correlated with standard atmospheric indices, such as 10.7-cm solar radiation or planetary indices reflecting particle fluxes in the atmosphere. The existence of a strong 285-day period in the acceleration however, is a clear indication that the effect, at least in part, is related to the geometry of the orbit with respect to the Sun and therefore of solar origin.

Recently, Anselmo *et al.*⁶ have investigated the effect of Earth albedo on Lageos and, in particular, variations bet-

ween the Northern and Southern Hemispheres. In their model, the force of the albedo as the satellite crosses the terminator of the Earth's shadow in the Northern Hemisphere is not balanced by the force of the albedo as the satellite crosses the terminator in the Southern Hemisphere because of the asymmetry of the albedo between the two hemispheres. When it is winter in the Northern Hemisphere, it is summer in the Southern Hemisphere. Moreover, the distribution of oceans and continents is different in each hemisphere. Anselmo *et al.*⁶ show that the asymmetry in the reflective properties of the Earth between the Northern and Southern Hemispheres can produce a variable acceleration of the Lageos satellite very similar to that observed. Over several years however, this effect averages to zero and therefore cannot explain the basic acceleration. Nonetheless, considering the simplicity of the model it seems likely that the north-south asymmetric albedo is the cause of the variations in the acceleration that are seen on Lageos. Further, the model predicts several other periodic components that are seen in the acceleration data.

J. Morgan⁷ has further suggested that asymmetries in the Earth's albedo in local time could cause the observed constant component of the acceleration of Lageos. In his model, the albedo of the Earth at dawn is different from the albedo at dusk, thus causing an acceleration (or deceleration) of the spacecraft. If the asymmetry in albedo with local solar time is preserved over extended periods of time, as the model suggests, then it could, in principle, provide the constant acceleration that is observed. Morgan's model has not yet undergone the testing required to establish the magnitude of the local time asymmetry that would be needed to sustain the acceleration.

Recent tracking data have suggested that the satellite is no longer accelerating but rather decelerating, causing the satellite orbit, at least temporarily, to stop decaying and expand. Verification of this change is extremely important because, if true, charge drag cannot be the only cause of the acceleration and an additional force, such as albedo, must be introduced. A temporary change in the albedo such that the acceleration becomes a deceleration is a much more acceptable explanation since the albedo is closely related to the vagaries of clouds and the weather and is known to vary geographically and seasonally. □

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Deep-Sea Drilling Project Leg 92

Advection in the East Pacific

from the Leg 92 staff

ADVECTION of seawater through oceanic crust probably continues for millions of years after the formation of the crusts at mid-ocean ridges. The history of advection is recorded in the alteration sequence of the crust itself, in the chemistry of hydrothermal exhalations and pore waters in deep-sea sediments, and in the hydrothermal component of the sediment column. Drilling and experiments conducted during Leg 92 of the Deep-Sea Drilling Project were designed as a multidisciplinary study of past and present hydrogeological processes that accompany the alteration of basalt by seawater. Nineteen holes were drilled at sites which form an east-west transect across the western flank of the East Pacific Rise at 19°S (see the figure). Borehole water samples were collected and several experiments conducted in Hole 504B, previously drilled through 1 km of basalt crust near the Costa Rica Rift during DSDP Legs 69, 70 and 83.

The best record of the alteration of the crust itself came from Hole 597C which was drilled in 25.8-Myr-old crust at the now extinct fast-spreading Galapagos Rise. At this site 91 m of basalts were drilled (with 48.5 m of recovery) which were olivine-poor to olivine-free ferrobasalts characteristic of fast-spreading ridges. The site was the most successful drilled on crust from a fast-spreading ridge, probably because of the high ratio of massive flows to pillow basalts.

A study of the sequence of vein and vesicle infilling and basalt alteration revealed that the crust had undergone several stages of fluid advection and concomitant alteration. The first stage was probably deuteric and is characterized by a pale-blue trioctahedral smectite. The second stage of alteration was non-oxidative and is characterized by a dark-green smectite, by pyrite and

native copper, and by other replacement minerals tentatively identified as celadonite, talc and chlorite. The final oxidative stage of alteration is dominated by calcite, oxyhydroxides and smectite.

One site, 600, was selected on the basis of high heat-flow values characteristic of advection in the hope that it would provide sediment samples whose pore waters showed evidence of advecting fluids. Unfortunately neither Site 600 nor any of the others revealed any unequivocal evidence of pore-fluid advection.

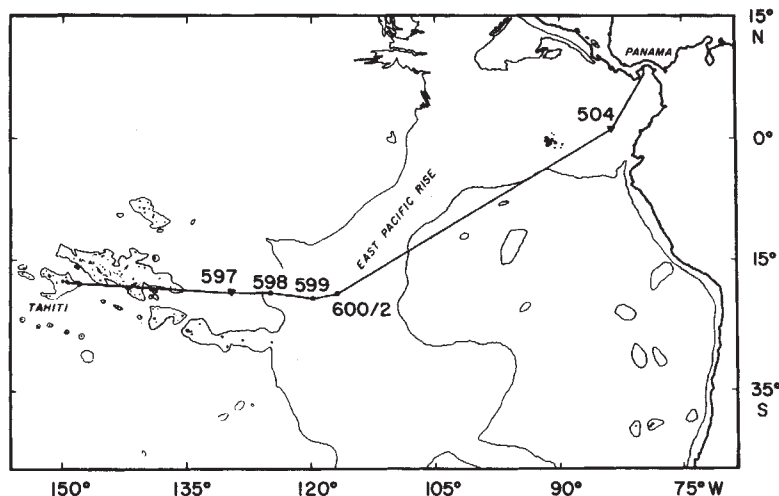
Evidence for advection of hot seawater through the crust is recorded in the sediment column. Clearly the sites accumulated hydrothermal phases while near the ridge crest at rates somewhat different from modern rates. The sediments at all the sites are dominated by calcareous nannofossil ooze with varying amounts of non-carbonate fraction which, except in the near-surface sediments of Sites 597 and 598, is made up of smectites, iron oxyhydroxides, amorphous material and reddish-yellow semi-opaque objects ('RSOs'). This assemblage has been found in other Pacific sediments and has been shown to be formed by the deposition and diagenesis of particulate matter of hydrothermal origin. The proportion of this hydrothermal assemblage increases downhole at all sites and a rough estimate of its mass flux based on the stratigraphy and physical properties of the sediments suggests that hydrothermal accumulation rates near the East Pacific Rise were greatest 4.5–8 and 21–25 Myr ago and that before the Pliocene, the hydrothermal accumulation rate was about twice that of the present. This accumulation could be controlled by differences in hydrothermal output at the ridge crest or by local to regional distribution processes.

A full understanding of advection requires detailed knowledge of the heat flow and permeability of the oceanic crust and their changes in time and space. It also depends on a knowledge of the chemical changes that occur when seawater and basalt react at various temperatures. These aspects of hydrothermal processes were examined when Leg 92 returned to Hole 504B, the deep basement hole near the Costa Rica Rift. Temperature profiles and water samples were taken while the borehole waters were still undisturbed. The former document the decreasing flow rate of water downhole to an underpressured aquifer in the uppermost 100 m of basalt. Since Leg 83 (November 1981), the rate has decreased from 25 m h⁻¹ to 2–3 m h⁻¹. The temperature at the bottom of the hole is 155–165°C.

The borehole waters provide a natural laboratory for the evaluation of reactions at elevated temperatures. Chemical analyses on board showed the linear relationship between decreasing Mg and increasing Ca when the latter is corrected for anhydrite precipitation using the sulphate concentration. The analyses further confirmed that the reaction is also balanced by Na and K which are removed during alteration reactions. Unfortunately, since the borehole was contaminated with bentonite drilling mud, many of the reactions could represent the alteration of bentonite as well as the alteration of basalt.

Several downhole experiment programmes addressed the relationship between crustal fracture density and distribution and hydrothermal flow. On a regional scale, an oblique seismic experiment was carried out in which the *Challenger* clamped a borehole seismometer in the hole while a second ship carried out both radial and circular shooting pattern. The experiment detected anisotropy from the seismic wave arrivals which appears to be related to crustal inhomogeneities.

On a smaller scale, a multi-channel sonic tool was used to study sonic velocities and waveforms in Layer 2A (which encompasses the underpressured zone). This work showed that the shape of the waveforms was related to differences in the amount of fracturing in the rock which had previously been detected by a borehole televiewer in the same interval. □



Map of the East Pacific Rise showing the positions of the drilling sites used in Leg 92.

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Immunology

Roving lymphocytes

from Ian McConnell

THE immune system is a roving bag of cells without a fixed anatomy. Unlike the nervous system it does not require a complex wiring programme for recognizing stimuli *in vivo* because lymphocytes are mobile cells which constantly traffic between and through lymphoid organs. The different traffic and localization patterns of lymphocytes *in vivo* raise questions on cell-cell recognition within a constantly mobile pool of cells. What is the nature of the interaction between lymphocytes and vascular endothelia? Are vascular endothelia specialized at different sites to permit different patterns of lymphocyte recirculation? What changes occur in endothelia at sites of chronic inflammation where lymphocytes leave the circulation in greater numbers? And is the failure of lymphocytes to penetrate certain tissues such as the central nervous system due to the absence of specialized endothelia? It is also unknown what factors control the partition and movement of lymphocyte populations and their subsets to domains within lymphoid tissue. In this issue of *Nature*, Gallatin, Weissman and Butcher¹ describe work in this field which represents new beginnings in the understanding of the molecular basis of lymphocyte recirculation.

During differentiation, lymphocytes acquire the characteristics which permit them to traverse specialized endothelia and enter lymphoid tissue^{2,3}. This selective interaction between mature lymphocytes and the specialized parts of the post-capillary venule known as the high endothelial venule (HEV) is seen in secondary lymphoid organs such as peripheral and central lymph nodes, gut-associated lymphoid tissue (for example Peyer's patches) and in extra-lymphoid sites of granuloma formation where the normal flattened endothelial cells change to resemble those of the HEV⁴. Lymphocyte recirculation through the spleen, which is also a secondary lymphoid organ, is organized differently and is not regulated by HEV.

The overall patterns of lymphocyte recirculation and by implication the nature of lymphocyte-endothelial cell interactions have been largely established by the lymphatic cannulation experiments of Gowans, Ford and their colleagues in the rat and Morris and his colleagues in sheep⁵. Whilst both systems have established some of the ground rules for lymphocyte recirculation, recent experiments using the cannulated sheep lymph-node model challenge the accepted view that lymphocyte traffic through lymphoid tissue is entirely random.

Lymphocytes isolated either from the efferent lymph leaving peripheral lymph or gut-associated mesenteric lymph nodes⁶,

or from the afferent lymph draining a granuloma^{7,8} and returned intravenously after radiolabelling to the same animal will preferentially recirculate through those regions from which they were first isolated. In these experiments lymphocytes are randomly mixed within the blood and selection at different tissue sites must be mediated at the level of the lymphocyte-endothelium interaction within different vascular beds of peripheral nodes, gut-associated lymphoid tissue and skin. In addition the antigen-specific selective mechanism operating on the traffic of antigen-reactive cells from blood into lymph nodes may also be explained by the presence of antigen on the luminal surface of endothelial cells^{9,10}. In this situation endothelial cells might be acting as antigen-presenting cells. Recent studies showing that lymphokines from activated T cells induce endothelial cells to express class II molecules of the major histocompatibility system, required for antigen presentation, further implicate endothelial cells in non-random lymphocyte traffic¹¹.

Evidence that the endothelial cells of the HEV contain a glycosylated macromolecule that may induce lymphocytes to cross the HEV from the blood further strengthens the idea that HEV-associated macromolecules play a part in lymphocyte traffic^{12,13}.

The first indication that lymphocyte-HEV interactions could be studied *in vitro* came from Woodruff and her colleagues^{14,15} who devised a novel quantitative assay which permitted the measurement of lymphocyte-HEV interactions through the adherence of lymphocytes *in vitro* to endothelial cells within sections of lymphoid tissue. Using this system they have shown that lymphocyte-endothelial cell adherence is both energy- and calcium-dependent, and mediated by surface determinants on lymphocytes that are sensitive to trypsin and appear to interact with the HEV. They have also described the purification from lymph of two soluble factors that regulate lymphocyte-endothelial cell adherence. One of these, a 160,000-molecular-weight glycoprotein, inhibits lymphocyte adhesion to HEV and is thought to be shed from the lymphocyte surface. Antisera to this molecule bind to recirculating lymphocytes and specifically block lymphocyte entry into lymph nodes *in vivo*¹⁶.

Further analysis of the molecular basis of lymphocyte traffic is described in this edition of *Nature* by Gallatin, Weissman and Butcher. Earlier work from this group has shown that murine lymphoma cells preferentially bind to either peripheral lymph-node HEV or Peyer's patch HEV but not both¹⁷. This correlates with findings *in vivo*⁶ that there may be sub-populations of

lymphocytes recirculating either through peripheral lymphoid tissue or through gut-associated lymphoid tissue.

To search for cell-surface molecules on those lymphoma cells which bind to separate HEVs Gallatin *et al.* have raised monoclonal antibodies to lymphoma cells that bind to peripheral lymph nodes. By screening on both types of lymphoma they have identified one monoclonal antibody (MEL 14) that specifically blocks the adherence of normal and lymphoma lymphocytes to peripheral-node HEV but not to Peyer's patch HEV and, moreover, specifically blocks lymphocyte traffic into peripheral lymph nodes but not to Peyer's patches *in vivo*. Immunoprecipitation studies with surface-labelled lymphocytes revealed a single 80,000-molecular-weight band in reducing conditions.

Surprisingly, normal lymphocytes which bind to Peyer's patch HEV also react with the antibody, though this does not inhibit adherence or traffic of these cells through Peyer's patch HEV. Their explanation for this is that lymphocytes express a family of 'receptors' for different 'acceptors' that vary in their distribution on endothelial cells. This interpretation does not fit with other data suggesting that there may be sub-populations of recirculating cells⁶⁻⁸.

The experiments of Gallatin *et al.* are notable for two reasons. First, they suggest that lymphocytes may possess recirculating phenotypes that can be defined by monoclonal antibodies. Such monoclonal antibodies may already be in existence and will be revealed by their anomalous reaction patterns on lymphocytes isolated from different parts of the lymphoid system. Second, they stress the value of considering leukaemias and lymphomas as malignant transformations of lymphocytes with particular recirculating phenotypes where the pathology can be understood in terms of the normal recirculation patterns of lymphocytes at different stages of differentiation. □

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Gut physiology

Trophic control of the intestinal mucosa

from Jared M. Diamond and William H. Karasov

It is a familiar fact that the mass of a muscle varies with use and disuse. Body-builders exploit this fact to develop particular muscles by exercising them, while conversely the muscles of bed-ridden patients or people with severed nerves atrophy. The mechanism of this trophic control is incompletely understood¹⁻⁴. Concealed inside us is another, less familiar trophic variation: the growth and atrophy of the intestinal mucosa. Current research on trophic control of the intestine combines basic studies of hormones, transport mechanisms and cell growth with clinical studies of patients recovering from intestinal surgery.

Under what circumstances does our intestinal mucosa receive more 'exercise'? Two familiar situations are pregnancy and lactation, both of which are associated with increased calorie requirements and food intake. Intestines of pregnant or lactating rats and hamsters can absorb more glucose, amino acids, zinc, copper and iron than can intestines of control animals⁵⁻⁷. This is made possible by increased absorptive area (increased intestinal length, villus height and mucosal area) resulting from stimulation of cell division^{6,8,9}. The intestine responds in a similar way to diabetes, which results in increased calorie requirements because of renal loss of glucose^{10,11}.

A factor common to these three conditions is increased feeding rate (hyperphagia), which may be necessary and sufficient to produce the responses. Rats induced to overeat by cold or by hypothalamic lesions also exhibit increased mucosal growth^{12,13}. Restricting the food intake of lactating or diabetic rats to normal levels prevents the mucosal growth that would otherwise occur^{14,15}.

A clinically important instance of trophic stimulation occurs after surgical resection of a diseased intestinal segment. Humans and rats with half of the small intestine excised maintain normal growth rates and absorptive capacities because an increase in mucosal mass and villus height in the remaining intestine compensates for the loss¹⁶⁻¹⁹.

When does our intestine receive less 'exercise'? Starvation is one such situation; the intestinal mucosa atrophies and absorption rates decrease. These effects are directly due to disuse of the intestine: rats nourished parenterally (that is, by intravenous infusion) for 7 days show no loss of body weight but do exhibit mucosal atrophy and reduced glucose absorption rates^{20,21}.

The various trophic responses of the in-

testine to use or disuse beg the question of the proximate signals. An intestinal cell has no direct experience of the fact that its owner is pregnant, nursing, starving, diabetic, or endowed with a surgically shortened gut.

The rat with total parenteral nutrition is a particularly convenient preparation for identifying the signals^{20,22-25}. Instilling nutrients into the intestinal lumen of parenterally nourished rats reverses the atrophy. The efficacies of different sugars and amino acids can be compared in order to elucidate the relative roles of their osmotic activity, affinity for transport mechanisms and metabolism as signals. Such observations are relevant to interpreting the proximal-to-distal decrease in mucosal mass, villus size, and sugar and amino acid absorption exhibited by the normal intestine. These normal anatomical and physiological gradients parallel the gradient in nutrient concentrations along the gut and may represent 'standing gradients of trophic response' to the luminal nutrient gradient. For instance, the anatomical and physiological gradients virtually disappear along with the nutrient gradient during parenteral nutrition. When distal segments of intestine are shifted more proximally by surgical transplantation or by resection of a medial segment, their villus size and glucose and amino acid transport rates increase to the levels seen in the intestinal segment normally found at that distance from the pyloric sphincter²⁶.

While the trophic effect of luminal solutes is largely direct, there is also evidence for an additional indirect effect mediated by hormones and/or nerves. Some of this evidence has been obtained by surgically isolating a loop of intestine, complete with its nerve and blood supply, from the intestinal canal and rejoining the severed proximal and distal ends of the canal. Nutrients infused into the main canal of parenterally nourished rats stimulate cell growth not only in the main canal but also, more weakly, in the bypassed loop, which has no direct contact with the nutrients²⁰. Similarly, amino acids infused into the distal intestine of parenterally nourished rats stimulate growth proximally as well as distally²⁷. Lactating animals or ones with resected intestines exhibit cell growth and enhanced transport in bypassed loops as well as in the main canal²⁸⁻³⁰. When only one member of a parabiotic pair of rats undergoes intestinal resection, intestinal cell growth is stimulated in both rats^{30,31}. Possible mediators of these indirect effects include nerves and various hormones such as

gastrin, glucagon, enteroglucagon, cholecystokinin, secretin, insulin and prolactin^{32,33}. The hormonal trophic effects on the main canal may in turn be partly mediated by the flows of pancreatic juice and bile that several of these hormones stimulate^{18,20,34}.

It is interesting to compare the obvious trophic effects of exercise on muscle with the less obvious effects of 'exercising' the intestinal mucosa. In both cases, at least part of the response depends on a system self-contained within the responding cell and not requiring other cells. Trophic responses of muscle can be obtained even after severing the motor nerve, by stretching the muscle or by producing muscle contraction through direct stimulation of the muscle membrane³⁵. Similarly, much of the trophic response of the intestinal mucosa appears to result from direct effects of luminal solutes on the absorbing cells. However, there are also indirect effects in both systems, mediated by motor nerves in the case of muscle, and by hormones, pancreatic and biliary secretions, and possibly nerves in the case of the intestine. In both systems the details of the proximate signals and the effector mechanisms underlying trophic effects are poorly understood. They constitute one of the most challenging issues in developmental biology. □

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Astrophysics

One step closer to the quasar power source?

from Martin Elvis

HAS the accretion disc in quasars been seen at last? Quasars have long been thought to be powered by the release of gravitational potential energy by matter falling onto, or into, a massive compact object¹. The compact object is commonly believed to be a black hole with a large mass¹ (10^7 – 10^9 solar masses, $M_\odot$). Just how this accreting matter meets its fate makes a great difference to the physics of how quasars work. Two basic paths have been investigated: spherical accretion, in which the material moves more or less radially inwards²; and disc accretion, in which the material has some angular momentum so that it forms a flat disc and spirals in³. Unfortunately, no observations have come close to deciding between the two alternatives. A fresh look at the data by Malkan⁴, however, now suggests that, at least in some quasars, there is indeed an accretion disc. Furthermore, using a simplified disc model, he is able to derive the mass of the central object (black hole?) and the rate at which matter is falling onto it. Remarkably he finds that in very luminous quasars, matter is being accreted at the maximum rate allowed by theory — the Eddington limit.

The paper is the second in a series to examine the ultraviolet (UV) excess in quasars. The optical continuum of quasars has been traditionally fitted by a power law⁵. In the last two or three years it has become clear that towards the UV the optical spectrum rises above the power-law value⁶. This excess is called the UV excess.

Early discussions of this excess^{5–7} were based only on conventional optical spectra both for defining the power-law slope and then for examining the excess. Malkan, in collaboration with W.L.W. Sargent⁸, was the first to realize that data from outside the normal optical range were important to understanding the UV excess. Their work included not only infrared (IR) data but also UV international ultraviolet explorer (IUE) data. This changed the picture. The IR (1–10 μm) power laws in quasars are typically much steeper ($\alpha = -1.0$) than the optical ($\alpha = 0$)⁹, and the use of the optical

power law begins to look artificial in this wider context. If the IR slope extends through the optical, the UV excess is far larger than had previously been considered (see the figure).

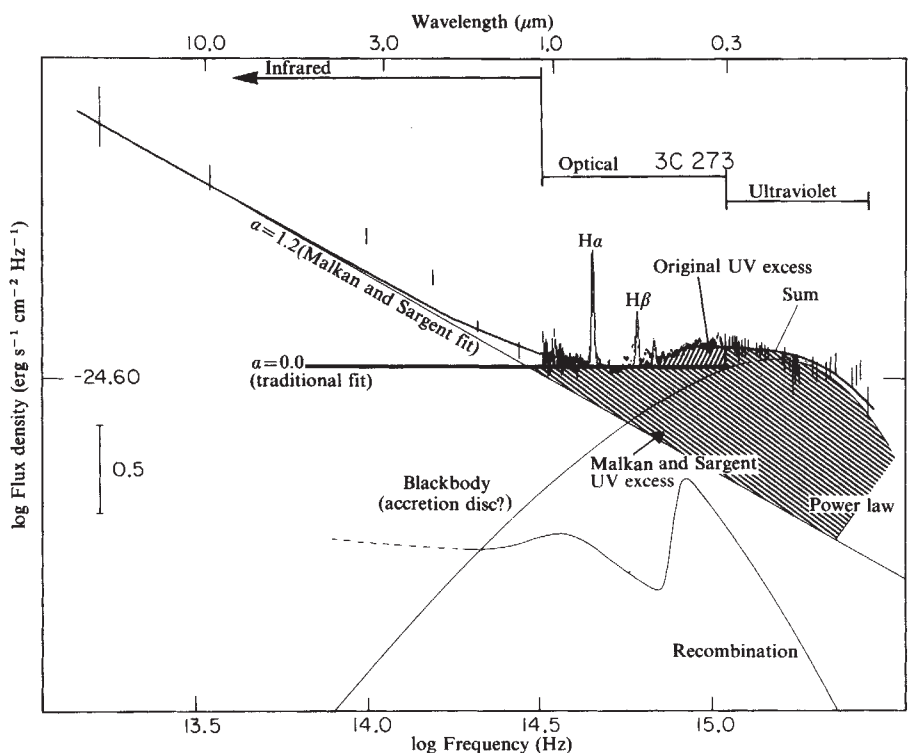
This new perspective suggested a new kind of fit to Malkan and Sargent. The blue bump might have two components — one being the expected Balmer continuum emission⁶ and the other a broader distribution that declined relatively slowly to the red, peaked around 2,000 Å and fell off rapidly in the UV. This shape made a black body a natural first choice for the second component. A single black body was an acceptable fit although the rapid fall-off in the UV, which would determine the temperature accurately, was not well constrained. The temperatures found were in the range 20–30,000K. Malkan and Sargent suggested that it is emission from an accretion disc. It is this possibility that Malkan investigates more closely in the second paper.

The limitation in defining the shape of the black body was the lack of UV data for wavelengths shorter than 1,200 Å. This deficiency could be made good only by observations on quasars at higher red-

shifts. These also had to be apparently bright, otherwise IUE could not detect them. Because of these two constraints, the quasars that Malkan observed were among the most luminous known — a point it is important to remember when considering the conclusions.

The result of the second paper is that the far-UV flux has too broad a distribution for a single black body, but that an accretion disc (which has a range of temperatures) would fit. The surprising aspect of accretion discs — at least for simple models — is that the resultant spectrum has only two free parameters: the mass of the black hole and the accretion rate. The observations constrain both parameters to strict limits and the two values are relatively independent. The extraordinary but satisfying point about Malkan's results is that they give large central masses ($\sim 10^9 M_\odot$) which are accreting at their Eddington limit. This is a theoretical maximum accretion rate, although it can be exceeded in special cases. So Eddington-limited accretion is a very plausible result for the most luminous quasars.

Malkan's results rest on the extension of the steep IR continuum and the assumption that it does not flatten as it goes into the optical. At the moment these are the least questionable assumptions that can be made but they do need to be tested. The 'non-thermal' nature of the IR emission could be supported by the detection of polarization in it. Even if a steep power law is a correct assumption the flat optical spectrum need not be black-body emission. Puetter *et al.*¹⁰



A combination of data from infrared, optical and ultraviolet telescopes shows a large excess above a power law. Malkan explains this in terms of an accretion disc radiating as a series of blackbodies at approximately 100,000 Kelvin. In the ultraviolet this dominates over the power law and the emission line spectrum.

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have put forward models with more than one non-thermal component. They use the synchrotron self-Compton mechanism as their basic process. Variability observations can be used to test these possibilities. The necessary multiwavelength simultaneous observing programmes are notoriously difficult to arrange and can be frustrating when the fickle objects of study

fail to perform on cue. We must work hard to find other ways to test Malkin's interpretation. Perhaps the few objects in which the UV excess is dominant will provide the key. □

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Immunology

Aetiology of autoimmunity

from Alfred D. Steinberg

It is very common for the sera of patients with autoimmune diseases to contain autoantibodies against several different tissues. It has however been difficult to determine whether the pattern of reactivity of the antibodies with different tissues reflects a range of autoantibodies with different specificities, or autoantibodies with the same specificity reacting with a common determinant on different tissues. This kind of question can be resolved, at least in part, by immortalizing large populations of B cells from patients with autoimmune diseases and analysing in detail the monoclonal antibodies they produce. One such study has recently been conducted by Haspel *et al.*, who report in this issue of *Nature*¹ that mice with a polyendocrine autoimmune disease induced by reovirus infection produce autoantibodies reacting with related or identical determinants in different tissues.

These results may have interesting implications for human autoimmune disease. For example, suppose patient A has a defect leading to the production of thyroid autoantibodies. Those antibodies happen to cross-react with gastric determinants. A defect in patient B leading to production of thyroid autoantibodies might result in molecules which can react with pituitary cells. Why should the specificities of the anti-thyroid antibodies produced by patients A and B differ? Several explanations are possible: patients A and B may, for example, have produced antibody in response to different antigenic determinants on the same structure; or they may have been immunized by different structures — modified 'self' determinants, viruses, bacteria and so on. Thus, although patients A and B both have anti-thyroid antibodies, their antibodies have different specificities and therefore have different cross-reactivities. As a result of cross-reaction with secondary organs, the anti-thyroid antibodies might also react with and induce damage in the secondary organ. Therefore, a similar abnormality leading to the production of anti-thyroid immunoglobulins in patients A and B might result in molecules which react with determinants in different secondary organs by virtue of the unique specificities of the antibodies. If the antibody molecules are capable of inducing damage in both the primary and

secondary organs, what might appear to be different diseases might, in fact, be quite closely related. The common involvement of the thyroid could suggest such a relationship. The common features of the illnesses of patients A and B would of course be harder to trace if the damage occurred only in the secondary organs.

A recurrent problem in the interpretation of studies with monoclonal antibodies is however that of cross-reactions. Reactivity of a single monoclonal antibody with apparently unrelated ligands has been demonstrated on several occasions²⁻⁵. A monoclonal autoantibody has been found to react with both human immunoglobulin and DNA-histone complexes⁶. Other monoclonal autoantibodies react with both DNA and cardiolipin, apparently by virtue of similar spacing of phosphodiester groups^{7,8}. Such findings help to explain some of the unusual properties of antibodies previously observed in patients with autoimmune rheumatic diseases; they also help to define the specificities of the antibodies. However, they serve only to complicate attempts to assess autoantibody heterogeneity in patients with autoimmune diseases. There is evidence that non-autoimmune organisms are capable of producing a range of monoclonal autoantibodies¹ whose production in harmful quantities is presumably normally suppressed by regulatory mechanisms⁹. This suggests that autoimmune animals may be expected to produce a heterogeneous range of autoantibodies. There is moreover evidence that the sample represented by antibodies from hybridomas may be atypical: for example, most anti-DNA molecules in the serum of patients are IgG but the anti-DNA hybridomas produced from such patients are preferentially IgM¹⁰.

It has been suggested that studies of monoclonal autoantibodies might yield information regarding the 'immunogen(s)' responsible for autoantibody production; for example in cases of immunization by self determinants modified by viral infection, or with a microbial agent which cross-reacts with self determinants. It is however possible that the autoantibody specificity in such cases will bear very little relationship to the 'immunogen'⁴, or represent only a small determinant on a larger structure

from which one could not easily deduce the whole¹¹. Finally, when autoantibody production reflects polyclonal B-cell activation primarily, and the specific expansion of subsets of B cells only secondarily, analysis of specificity may bypass certain critical pathogenetic mechanisms⁹.

Despite their limitations, studies of monoclonal autoantibodies force us to re-examine our basic concepts of autoimmune diseases. The paper by Hazel *et al.*¹ provides clues to a unifying concept of the pathogenesis of multiple endocrine autoimmune disorders. □

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100 years ago

From *Nature* **28**, 12 & 19 July 1883.

CHOLERA PROSPECTS

THE early history of cholera is involved in a good deal of obscurity, and it was not until 1817, when the disease caused a terrible mortality amongst our troops in India, and subsequently spread into different parts of the Asiatic continent, that any noteworthy attention was given to it by European observers. It is very possible that even previous to the present century cholera had made its way into Europe, but the first trustworthy record of its course westwards was in 1831, when it travelled by way of Russia and the Baltic, and, as far as we know, made its appearance for the first time in England. In 1866 the disease became epidemic in the metropolis, and its special incidence in the East End was shown to be in the main due to the polluted character of the water delivered to that part of London. The disease is once more prevalent in Egypt; it has already caused over 2000 deaths in a few towns in the delta of the Nile, and the prospect of its spread to the several ports of Europe is regarded with universal concern.

The etiology of cholera, in so far as relates to its influence in this country, does not admit of much doubt. The infection must be actually imported into our midst; it has never yet been imported except through human agency. In all essential respects the disease appears to spread under much the same conditions as favour the spread of enteric or typhoid fever, and, like that disease, it has in this country mainly been associated with the use of water supplies, which have been subjected to the risk of receiving the specific infection. What that infection consists in is not yet known, but judging from analogy it is a definite organism capable of reproducing its own kind under those conditions of filth which we have adverted to as being associated with the spread of the disease. In the case of anthrax, which causes the so-called wool-sorter's disease in man, and in the case of relapsing or famine fever, the microscope has succeeded in showing the organisms which lead to the production of those specific affections; but in the case of cholera no such results have as yet been attained, and this notwithstanding the laborious microscopic and other researches which have been made in India and elsewhere.

M. Pasteur has been appointed head of the Sanitary Commission formed in Paris in view of the dreaded visitation of cholera.

A French scientific periodical puts forward the idea of a joint occupation of Mecca by the several European powers for the purpose of stopping pilgrimages thither and thereby preventing the further dissemination of cholera through the crowding of people in so pestilential a city, especially when the Ramadan falls in summer.

Lunar-solar periodicities of large earthquakes in southern California

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Large earthquakes in southern California with epicentres between 33 and 36°N have statistically significant 12-hourly, lunar fortnightly and 18.6-yr periodicities. Smaller earthquakes in the same region do not display these periodicities. A search for tidal effects associated with these periodicities shows that large earthquakes have significant correlations with the times and orientations of daily/semi-daily tidal stresses while the lunar fortnightly term is associated with the ocean tides along the southern Californian coast.

MOST of the presumed correlations of earthquake occurrence with actual periodicities that have appeared in the literature¹⁻³ can be shown to be spurious⁴, or due to the failure to apply proper statistical scrutiny^{5,6}; popular suppositions of such correlations are usually due to uncritical application of a *posteriori* reasoning. Nevertheless, the possibility that the Earth tides might trigger earthquakes has remained an attractive postulate even though tidal stresses are very small compared with the stress drops in earthquakes; the Earth tides are the largest known sources of oscillatory stresses with relatively short periods. In many cases correlations with the Earth tides are masked by populous aftershocks; aftershocks are more easily correlated with their large parent earthquakes than with the tides^{7,8}. Conversely, failure to take aftershock clustering into account adequately may lead to apparent correlations with the tides⁹. To date, most of the systematic searches for correlations, that involve a removal of aftershocks, have been shown to be barren.

A study of large earthquakes may minimize the effects of aftershocks, under the assumption that large earthquakes are not as likely to be correlated events, that is aftershocks of other strong events. Searches for correlations of times of large worldwide earthquakes with the obvious periodicities have also proved to be unprofitable^{10,11}. However, Klein has shown that there is a significant tendency for earthquakes in selected regions of the world to occur at times at which the daily tidal stresses are so aligned as to provide maximum enhancement of the stresses associated with plate tectonics¹². Heaton has shown that large shallow-focus earthquakes worldwide appear to be correlated with tidally generated diurnal shear stresses, but only if the earthquakes do not have a strike-slip focal mechanism¹³; Heaton speculates that earthquakes with magnitudes >3 are more likely to be triggered by the tides than are small events, but he has not tested this hypothesis.

We now report our investigations into the correlation of large earthquakes with directional tidal stresses as well as lunar and solar periodicities for earthquakes in one, narrowly defined, geographical region, thereby combining selected features of earlier investigations. We consider earthquakes in southern California in a finite range of epicentral latitudes. Our major contributions are to eliminate aftershock events from the correlation analysis, and to see if there are different correlations for strong earthquakes than for weaker ones.

The San Andreas Fault and other major faults in this region extend in a direction that is roughly NW-SE. Since motions on these faults are right-lateral strike-slip, extensional tidal stresses in the east-west direction both unload the normal stresses and increase the shear stress that tends to produce faulting (Fig. 1a). (If the motions on the fault had been left-lateral, east-west

extensional stresses would unload the normal stresses but the shear component would oppose the faulting. Thus, correlations of earthquakes with the tides might be expected to be absent for a worldwide sample if the combination of faulting directions and fault orientations were random.) Since east-west extensional stresses are maximized at moonrise/set and sunrise/set for lunar and solar tidal influences taken separately, we can hope to find correlations with times of solar or lunar day; these should be maximized at times of full and new moon, when the lunar and solar rising/setting times are near one another. Almost 50 yr ago, Allen¹⁴ investigated the times of 1,200 earthquakes on or near these NW trending faults of southern California and found a large but not statistically significant component of periodicity with fortnightly period.

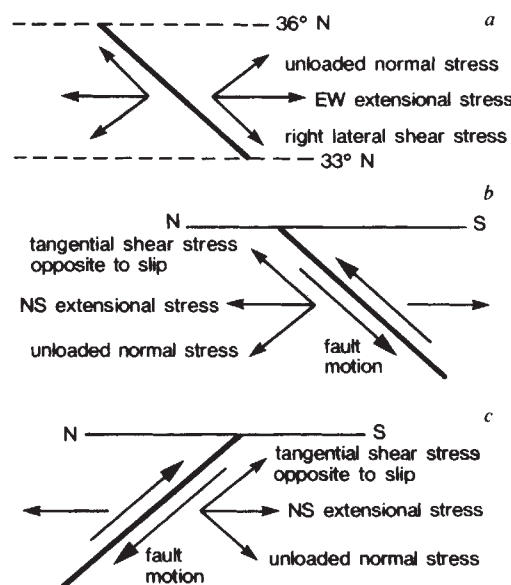


Fig. 1 a, An east-west extensional stress on a strike-slip fault striking N 45° W, has stress components that produce maximum unloading of the normal stress and maximum tangential shear stress in the direction of fault motion. One-half period later, the stresses are reversed. b, A north-south extensional stress on a dip-slip fault, dipping 45° S, causes unloading of the normal stress and a tangential shear stress opposite to the presumed direction of fault motion. c, A north-south extensional stress on a dip-slip fault, dipping 45° N, causes unloading of the normal stress and a tangential shear stress opposite to the presumed direction of fault motions.

Table 1 List of earthquakes

Date	Time (PST)	Lat. (°N)	Long. (°W)	Mag.	Focal mechanism
Mag. ≥ 6.0					
9 January 1857	0800	35.00	119.00	>8.0	S
21 April 1918	1432	33.75	117.00	6.8	S
22 July 1923	2330	34.00	117.25	6.25	S
29 June 1925	0642	34.30	119.80	6.25	S
10 March 1933	1754	33.62	117.97	6.3	S
25 March 1937	0849	33.41	116.29	6.0	S
15 March 1946	0549	35.73	118.05	6.3	D
10 April 1947	0758	34.98	116.55	6.2	D
4 December 1948	1543	33.93	116.38	6.5	S
21 July 1952	0352	35.00	119.02	7.7	D
19 March 1954	0154	33.28	116.18	6.2	S
8 April 1968	1828	33.19	116.13	6.4	S
9 February 1971	0600	34.41	118.40	6.4	D
Mag. < 6.0					
2 October 1933	0110	33.78	118.13	5.4	
31 May 1938	0034	33.70	117.51	5.5	
17 May 1940	2103	34.08	116.30	5.4	
30 June 1941	2350	34.37	119.58	5.9	
14 November 1941	0041	33.78	118.25	5.4	
28 August 1943	1945	34.27	116.97	5.5	
22 December 1943	0750	34.33	115.80	5.5	
12 June 1944	0316	33.99	116.71	5.3	
1 April 1945	1543	34.00	120.02	5.4	
15 August 1945	0956	33.22	116.13	5.7	
8 January 1946	1054	33.00	115.83	5.4	
18 July 1946	0627	34.53	115.98	5.6	
24 July 1947	1410	34.02	116.50	5.5	
2 May 1949	0325	34.02	115.68	5.9	
28 July 1950	0950	33.12	115.57	5.4	
12 January 1954	1533	35.00	119.02	5.9	
16 December 1955	2207	33.00	115.50	5.4	
28 January 1961	0012	35.78	118.05	5.3	
28 April 1969	1520	33.34	116.35	5.8	
12 September 1970	0630	34.27	117.54	5.4	
21 February 1973	0645	34.06	119.03	5.9	
25 February 1980	0247	33.50	116.51	5.5	

But Allen did not remove aftershocks systematically and hence the result could be biased, even to the possible elimination of the effect. We seek correlations of the times of large, shallow strike-slip earthquakes with the combination of diagonal terms of the strain tensor ($4e_{EW} + e_{NS}$) which is proportional to the east-west stresses for a Poisson solid for shallow earthquakes.

Some strong earthquakes occur with dip-slip motions on faults that strike obliquely to the NW-SE trend with a large variation in strike; we can take the displacements in these earthquakes to be consistent with the relative motions of the Pacific and North American plates. For simplicity we assume that the mean strike is east-west. For dip-slip faulting on shallow faults striking east-west and dipping 45° to the north or south, both the unloading normal stresses and the shear stresses along the plane for motions in the direction of dip are proportional to $(4e_{NS} + e_{EW})$ for a Poisson solid. Unfortunately, maximum tidal unloading of the normal stress on faults with these orientations occurs at the time of minimum tidal shear stress and vice versa (Fig. 1b, c), so the issue is not as clear-cut as in the case of strike-slip faulting along faults that strike N 45° W. Allen¹⁴ assumed that the N 45° W regional trend dominated the seismicity, but was unable to find a significant correlation. We take both of the above two models of fault orientation into account as appropriate.

The diurnal and semi-diurnal strain tides have large fortnightly amplitude modulation effects as well as large phase shifts (Fig. 2). The phase shifts, if not taken into account, would lead to misidentification of the times of peaks of tidal components. In the cases of both the diurnal and fortnightly extrema of the tides, we refer the occurrence of earthquakes to the times of these extrema, rather than to perfectly periodic occurrence.

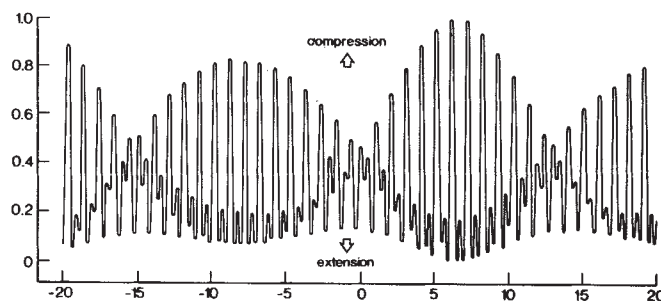


Fig. 2 Strain component $4e_{EW} + e_{NS}$ for the earthquake of 29 June 1925. The time scale extends from 20 days before the time of earthquake to 20 days afterward. The origin is at the time of the earthquake. Vertical scale is normalized to unit maximum fluctuation over the 40-day interval.

List of earthquakes

We restrict our attention to earthquakes with epicentres within the southern California region¹⁵ between latitudes 33° N and 36° N, for the period of instrumental recording 1933–80, with magnitudes $M \geq 5.3$ (refs 16, 17). Identifiable earthquakes occurring within a few weeks of other large events were deleted from the list, whether the large events were inside or outside the region. Thirty-one earthquakes remain on our list, of which 22 are in the range $5.9 \geq M \geq 5.3$ and 9 are in the range $M \geq 6.0$ (Table 1). To increase the number of large earthquakes on which the hypothesis will be tested, we have added four pre-1933 events from the historical catalogue¹⁸ having nominal epicentres well within the above region, nominal magnitudes that are identifiably >6.0 , and enough accuracy in time, magnitude and especially location, that they probably represent temporally unbiased additions to our data set.

Temporal correlations

Figure 3a displays the local time of occurrence of the earthquakes in our list, modulo 12 h. The smaller events ($M < 6.0$) are more-or-less uniformly distributed, while the large magnitude events seem to cluster about 6 a.m./p.m. local time (PST). These times are an approximation to sunrise/sunset for simplicity. Clustering is strongly suggested. Of the 13 large events with $M \geq 6.0$, 10 are in the central half of the diagram; we apply a statistical test to determine whether the clustering is significant.

A similar diagram (Fig. 3b) is obtained for the times of occurrence of these same earthquakes plotted relative to the 18.613 yr periodicity of the precession of the line of nodes of the Moon's orbit. When the phase is zero, the lunar declination reaches its greatest northern extent; one zero of the phase is 12 January 1932¹⁹. As before, most of the large earthquakes ($M \geq 6$) seem to cluster about the time of zero phase, while the smaller ones ($M < 6$) appear to be uniformly dispersed. We did test for the significance of the apparent clustering.

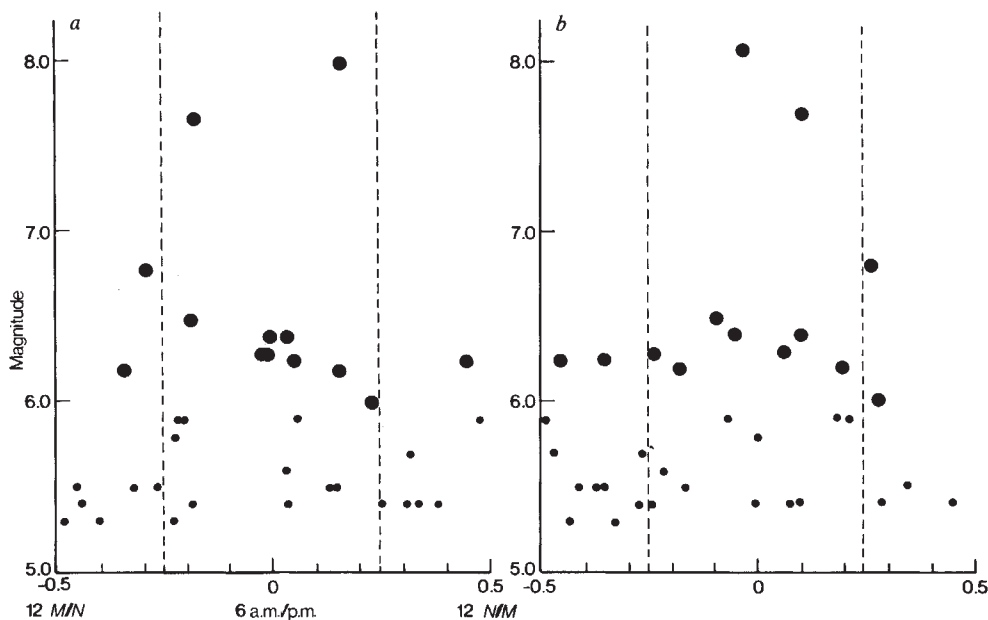
The times of most of the large earthquakes also seem to cluster around the times of new and full Moon, while the smaller ones are not clustered. The diagram for this case is not presented.

Although the number of large earthquakes is too small to support an analysis with several degrees of freedom, a statistical procedure for the verification of the presence of clustering can be formulated which evaluates the significance of a cyclic Poisson process in comparison with the null hypothesis of a homogeneous Poisson process²⁰. By hypothesizing a cyclic behaviour with a known phase shift, the number of degrees of freedom in the analysis can be reduced to one.

We accept the null hypothesis at the 95% confidence level ($\nu = 1$) if

$$\chi^2 = \frac{2}{N} \left(\sum_{i=1}^n \cos \omega_0 t_i \right)^2 < 3.84$$

Fig. 3 *a*, Times of occurrence of southern California earthquakes on a scale of period 12 h; 6 a.m./p.m. is at the centre of the diagram. Large earthquakes cluster in the central half of the diagram. *b*, Times of occurrence of southern California earthquakes on a scale of period 18.613 yr. The zero of phase is 12 January 1932. Large earthquakes cluster in the central half of the diagram.



where N is the total number of events, $T = 2\pi/\omega_0$ is the period of the cyclic process and t_i is the time of occurrence of the i th earthquake relative to the time of anticipated maximum correlation. We accept at the 90% confidence level if $\chi^2 < 2.71$. For the cases of the 12-h variations, the zero of time is taken to be either 6 a.m. or 6 p.m. LT; for the fortnightly variation, the zero is taken to be either new or full Moon and we use unit periodicity for the time between nearest full and new Moons; for the 18.6-yr variation, the zero is as above.

In the first column of Table 2 we consider all 13 strong events with $M \geq 6.0$. In the second column, we restrict the strong earthquakes to those with $M \geq 6.3$; because magnitudes are not always accurately determined, we thus avoid contamination of the set of large earthquakes by events that might have improperly assigned magnitudes. In the third column, we consider shocks after 1932, and avoid questions of the accuracy of determinations of magnitudes or locations of historical earthquakes. Shocks of smaller magnitudes are considered in the last two columns. In the second of these, we add the five shocks deleted from the list of strong earthquakes in the second test.

For none of the semi-daily, lunar fortnightly or 18.6-yr periodicities do the smaller earthquakes show any significant correlations. On the other hand, we can reject the hypothesis of absence of clustering of large earthquakes with fortnightly lunar phase and with the rise and set of the Sun for all three selections of large earthquakes at the 95% level. We reject the null hypothesis at the 95% level for the correlation of the strongest earthquakes ($M \geq 6.3$) with the 18.6-yr precession of the lines of nodes of the Moon's orbit; rejection is at the 90% level for $M \geq 6.0$.

Surprisingly there is no correlation of large earthquakes with the rise and set of the Moon; the lunar tide is considerably stronger than the solar tide, and if a correlation is found with the rise and set of the Sun, then should not, at least, an equally strong correlation be found with Moon rise/set?

Not unexpectedly, we accept the null hypothesis regarding an annual term with zero phase at the winter solstice (the annual tides are small) and regarding the periodicity of the lunar perigee phase. The latter is the pure fortnightly-bifortnightly term, which is small since the eccentricity of the lunar orbit is only $\sim 5\%$. The phases of the Moon correspond to the fortnightly modulation of the Earth's daily tide, which is a large effect.

The null hypothesis can be rejected at the 95% confidence level for the correlation of the earthquakes with $M \geq 6.0$ with an annual term with phase as a parameter; the phase of maximum correlation is close to the vernal equinox. (In this

Table 2 χ^2 analysis for cyclic Poisson process*

No. of events	13	8	9	22	27
Magnitude range	≥ 6.0	≥ 6.3	≥ 6.0	5.3–5.9	5.3–6.25
Dates	1857–1980	1857–1980	1933–1980	1933–1980	1923–1980†
Lunar phase‡	4.71	5.02	8.49	1.23	0.51
Time of day§	3.94	6.32	5.20	0.49	0.39
Precession phase	2.95	7.09	5.58	0.28	0.54
Lunar perigee phase	1.17	1.54	3.78	0.34	0.20
Lunar rise/set time¶	0.44	2.23	0.35	0.03	0.04
Day of year#	0.13	0.39	0.05	0.46	1.45
Normalized time of day and lunar phase	5.60	7.62	8.77	0.62	0.33
Day of year (2 d.f.)**	6.45	4.57	7.64	0.50	2.09

* A homogeneous Poisson process can be rejected if the entry is greater than 3.94. Entries are the values of $(2/N)(\sum \cos \omega_0 t_i)^2$.

† Earthquakes of 1923, 1925 are added to list of post-1933 earthquakes.

‡ Period = $\frac{1}{2}(29.53)$ days, zero phase = full/new moon.

§ Period = 12 h, zero phase = local 6 a.m./6 p.m.

|| Period = 18.613 yr.

¶ Period = 12.4 h.

Zero phase = 21 December.

** A homogeneous Poisson process can be rejected at the 95% level if the entry is > 5.99 , and at the 90% level if the entry is > 4.60 .

case, for $\nu = 2$, $\chi^2 > 5.99$.) Curiously, the strongest earthquakes ($M \geq 6.3$) are only correlated at the 90% confidence level.

We have considered the correlation of large earthquakes jointly with time of day and lunar phase. The latter parameter is the distance of the earthquake time from the point 6 a.m./p.m. and new/full Moon, properly normalized. As both components are separately nonrandom at the 95% confidence level, it is not unexpected that strong earthquakes are nonrandom using both parameters jointly.

We conclude that there are indeed significant nonrandom correlations of the occurrences of strong earthquakes in this latitude range, but not weak earthquakes, with some of the solar-lunar times.

Tidal correlations

We search for correlations with tidal processes to promote the notion that the above statistical results have a physical basis. We have computed the tidal strains ($4e_{EW} + e_{NS}$) and ($4e_{NS} + e_{EW}$) due to the gravitational attraction of Sun and Moon for 20 days before and 20 days after each earthquake, at the epicentre of each earthquake. If the Earth tides trigger

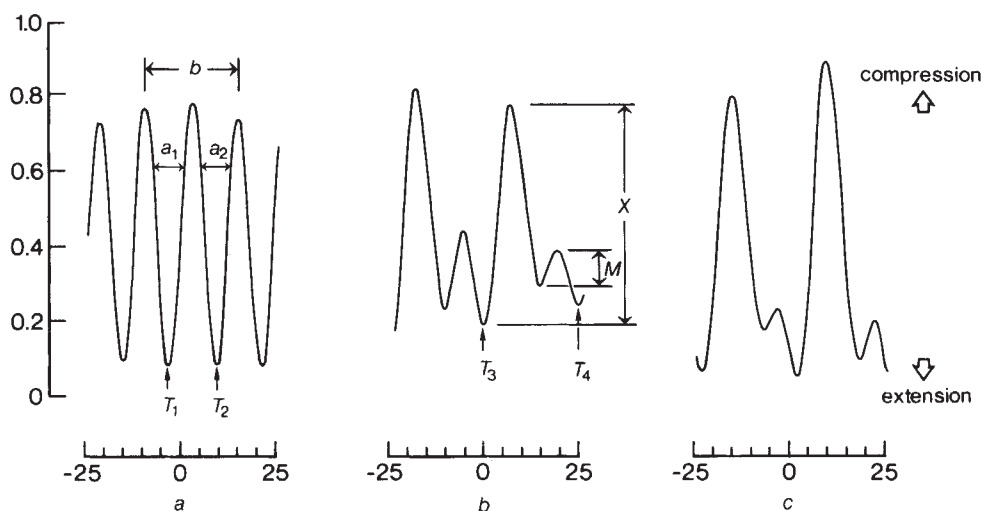


Fig. 4 *a*, Tidal strain component $4e_{NS} + e_{EW}$ for the earthquake of 25 March 1937. The time scale extends from 25 h before to 25 h after the earthquake. Vertical scale is normalized to unit maximum fluctuation over a 40-day interval spanning the event. *b*, Tidal strain component $4e_{NS} + e_{EW}$ for the earthquake of 10 April 1947. Scales defined as in *a*. *c*, Tidal strain component $4e_{EW} + e_{NS}$ for the earthquake of 23 July 1923. Scales defined as in *a*.

earthquakes, then earthquakes on strike-slip faults would be more likely to occur near the times of fortnightly maximum extension of ($4e_{EW} + e_{NS}$) than at the times of maximum peak-to-peak of this quantity; the two times differ in many cases by a day or more.

We define a relative fortnightly tidal time of occurrence of an earthquake to be the elapsed time since the preceding extremum in units of the time between the extrema spanning the earthquake. We have made many searches for correlations between the times of extrema of various components of the tidal strains, and the occurrence of large earthquakes. The probability of rejecting a homogeneous Poisson process as the null hypothesis is $<90\%$, for all combinations of fortnightly Earth-tidal strains that we have considered.

The problem of diurnal correlations is rather more complex. The tidal time function has large variations in shape from day to day, which depend on the relative amplitudes of the 24- and 12-h terms (Fig. 4). In one case we have used as our measure the fractional part of 24 h for which the extensional strain is greater than the strain at the time of the earthquake, in the period ± 12 h before or after the largest tidal compressional strain in the 24-h period spanning the earthquake; this is the ratio $(a_1 + a_2)/b$ in Fig. 4*a*. Neither the stronger nor the weaker earthquakes are correlated according to this procedure.

Other models such as the diurnal time fraction from the previous maximum 24-h compressional stress, the previous maximum 24-h extensional stress, and other variations have been considered. In only one do we find well-defined 24-h or 12-h periodicities.

In the case of rejection of the null hypothesis that we have identified, we have assigned *a priori* a focal mechanism to each of the strong events from one of the two categories above. These identifications are indicated in Table 1 by letters S and D for strike-slip faulting on a NW-SE fault and dip-slip faulting on an east-west fault respectively. A second assignment refers to the variable shape of the tidal strain curves over a 24-h interval. In Fig. 4*b*, we let M be the tidal strain interval between the lesser maximum tide and the greater minimum tide in a 24-h interval between the least minima spanning the earthquake; the latter occur at times T_3 and T_4 . We let X be the tidal strain interval between the greatest maximum tide and the least minimum tide in the same 24-h interval. If the ratio M/X is $>1/3$, we make the correlation with the semi-diurnal tide (for example with the times (T_1, T_2) in Fig. 4*a*), while if the ratio is $<1/3$, correlation is made with the diurnal tide (for example with the times (T_3, T_4) in Fig. 4*b*). The threshold $1/3$ was a quantity chosen *a priori*. Under these two *a priori* assumptions, we find a correlation with the times of the occurrence of large earthquakes at the 95% level of rejection of the null hypothesis. Our reasons for choosing this rather convoluted pair of criteria are to take into account, at least qualitatively, variations in the temporal shape of the stress field and variations

in the spatial orientation of earthquake faults, in trying to parameterize the triggering stresses. In any case, the choices were made *a priori*. The less contrived models have been tested and found to lead to failure to reject the null hypothesis.

With regard to the fortnightly correlation, the null hypothesis cannot be rejected for any of the Earth tide models we have investigated. The only case of rejection of the null hypothesis on the fortnightly time scale that we have found is with the times of high oceanic tides at Los Angeles harbour. Rejection in this case is at the 90% level. The ocean tides are well correlated with the lunar phases, while the local Earth tides are not.

Conclusions

We began this investigation with a well-defined physical model in mind, namely one which focused on the interaction of lunar-solar extensional Earth tides in a part of the Earth where strike-slip faulting occurred with a particular geographical orientation. While we have found statistically significant correlations of large earthquakes with unbiased astronomically-defined temporal effects, in almost all cases, we find that triggering due to the Earth tides is not apparent. In one case, however, we have found a triggering by the Earth tides on the diurnal-semi-diurnal time scale if we take fault orientation and phase shifts into account. We have also found a correlation of times with the fortnightly ocean tides along the Pacific Coast. Smaller earthquakes are not correlated with the lunar-solar times. With regard to the temporal correlations, we announce these nonrandom effects at this time as clear-cut but, nevertheless, a subject of some curiosity, especially since the correlation is absent with regard to the times of Moon rise/set.

It is of interest to extrapolate from these observations to anticipate the time of the next episode of high potential for the occurrence of large earthquakes in southern California. The next time of maximum lunar declination is November 1987. A literal extrapolation of our observations implies that during a window of a few years width astride this date, at times near full or new Moon, and near sunrise or sunset, one might be more likely than otherwise to observe one or more large earthquakes in southern California. We refrain from giving these remarks greater credence because we cannot evaluate the variances of likelihood estimates for what is, at present, only a fit to a heuristic physical model, buttressed with only a few data.

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Pb, Sr, Nd and Hf isotopic evidence of multiple sources for Oahu, Hawaii basalts

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Pb, Sr, Nd and Hf isotopic compositions of Oahu volcanics indicate that the three principal volcanic series on Oahu—Koolau, Honolulu and Waianae—were derived from isotopically distinct sources. Honolulu and Waianae basalts plot on the Nd–Pb–Sr ‘mantle plane’ whereas Koolau data plot distinctly below the plane.

THE island of Oahu belongs to the Hawaiian volcanic chain and is situated 210 km north-west of the presently active island of Hawaii in the Pacific. The island, 35 × 70 km, is structurally and petrographically subdivided into three main volcanic suites which are in order of eruption, the Waianae, Koolau and Honolulu volcanic series. Bargar and Jackson¹ calculated that Waianae is the larger shield volcano of the island with a volume of 25,000 km³ followed by the Koolau volcano with 21,000 km³. Conventional K–Ar age determinations by Doell and Dalrymple² indicate that the Waianae volcano was formed ~3.6 Myr ago and the Koolau volcano ~2.55 Myr ago. The Honolulu series is much younger, having erupted ~0.6 Myr ago³.

The eruptive sequence of the Waianae volcano is tholeiite followed by late-stage alkalic rocks. The Koolau volcano apparently produced only tholeiitic lavas⁴. The post-erosional Honolulu volcanic series, consisting of melilite nepheline basalts, nephelinites and alkali-olivine basalts^{4–6}, erupted through and is scattered over the southeastern part of the Koolau tholeiitic shield.

To obtain more information about the source region of the Oahu basalts Sr, Nd, Hf and Pb isotopic studies on the three well-defined rock-suites have been performed. Sample locations for Honolulu and Koolau basalts are summarized in Table 1. Sample locations for Waianae basalts are given by Macdonald and Katsura^{5,6} and Lanphere *et al.*⁷.

Results

The Sr, Nd, Hf and Pb isotopic data for four nephelinites and two alkali-olivine basalts of the Honolulu series, five tholeiites of the Koolau series and three tholeiites and three alkali basalts of the Waianae series are given in Table 2. No age correction was necessary for any of the isotopic data. The most striking feature of the investigation is the significant difference in

isotopic compositions, most evident in Sr, among the Waianae, Koolau and Honolulu volcanics. These results agree with earlier studies by Powell and Delong⁸ and Lanphere and Dalrymple³ who reported differences in ⁸⁷Sr/⁸⁶Sr between Koolau and Honolulu basalts.

Nd and Hf isotopic compositions are also distinctly different for each group. The alkali and nepheline basalts of the Honolulu series are the most radiogenic in Nd and Hf isotopes, showing ϵ_{Nd} values between +7 and +8 and ϵ_{Hf} values between +12 and +16. The least radiogenic samples with respect to Nd and Hf isotopes are the tholeiites of the Koolau volcano, showing ϵ values of +1 to +2 and +5 to +8, respectively. The isotopic compositions of the alkali and tholeiite basalts of the Waianae volcano are again intermediate between Honolulu and Koolau with ϵ values of +6 to +7 for Nd and +9 to +13 for Hf. The three groups with distinctly different isotopic compositions may imply three distinct sources, a single grossly-heterogeneous source, or mixing between two sources.

Pb isotopic compositions fall into only two groups: the Koolau tholeiites with ²⁰⁶Pb/²⁰⁴Pb = 17.8–18.0 and the Honolulu and Waianae basalts with ²⁰⁶Pb/²⁰⁴Pb = 18.0–18.2. Our Pb data for Honolulu basalts are in agreement with those of Sun⁹ who obtained a mean ²⁰⁶Pb/²⁰⁴Pb ratio of ~18.16 for six Honolulu samples. The seemingly identical Pb isotopic compositions for Waianae and Honolulu basalts are quite surprising in view of the data from the other isotope systems. If the isotopic characteristics of Waianae do in fact reflect mixing of the Honolulu and Koolau sources, then compared with Sr, Nd and Hf, Pb must have been more enriched in the Honolulu source than in the Koolau source.

Clague and Frey¹⁰ have shown that, although the Nd isotopic characteristics of Honolulu volcanics require a net depletion of Nd/Sm through geological time¹¹, the trace-element characteristics of Honolulu basalts suggest that they were derived from a trace element (and Nd/Sm) enriched source. They attributed this discrepancy to recent metasomatism of the Honolulu source. Thus, the Pb isotopic compositions of the Honolulu basalts and the apparent lack of correlation between the Pb isotopic compositions and those of Sr, Nd and Hf among

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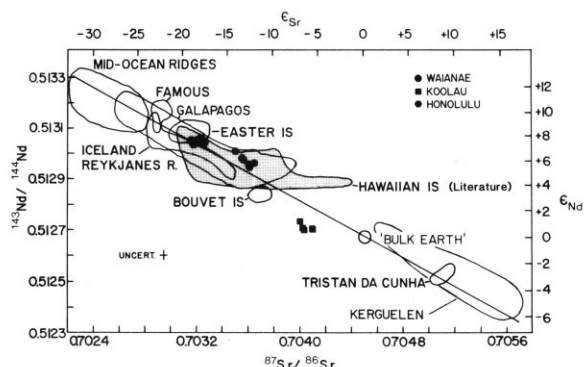


Fig. 1 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{143}\text{Nd}/^{144}\text{Nd}$ for basalts from Oahu compared with MORB, Iceland and Reykjanes Ridge, Ascension, Galapagos, Tristan da Cunha, Bouvet, DSDP Leg 37 and other Hawaiian data³¹⁻³⁹. The mantle trend is shown by the solid line.

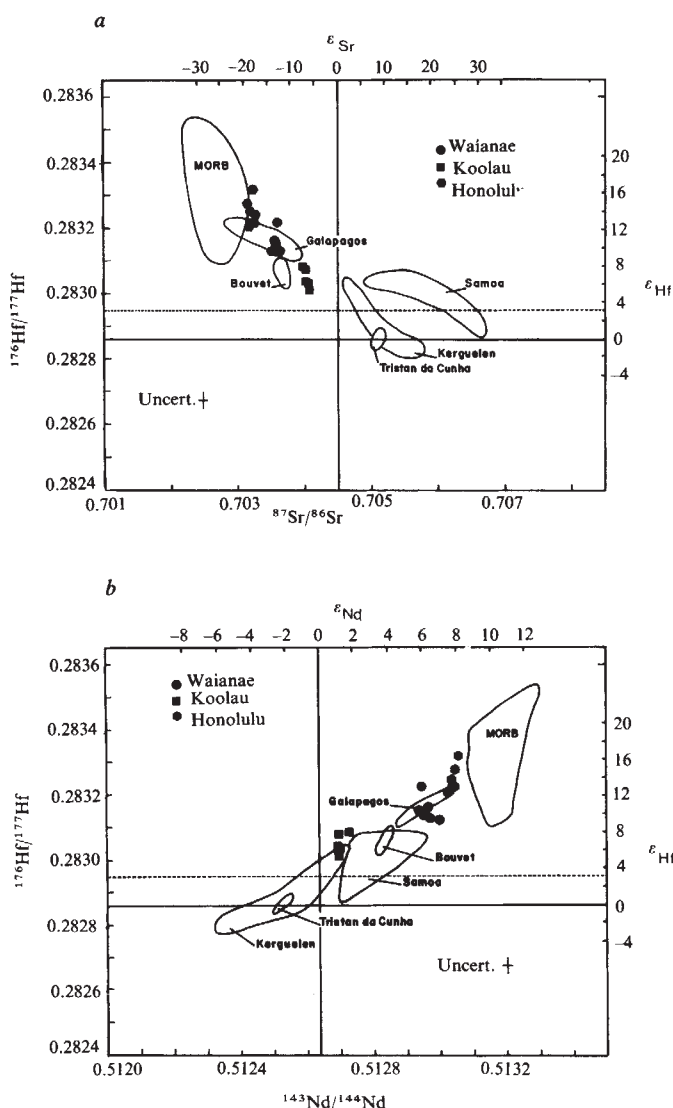


Fig. 2 *a*, $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{176}\text{Hf}/^{177}\text{Hf}$ for basalts from Oahu compared with other ocean island basalts and MORB^{40,41}. *b*, $^{143}\text{Nd}/^{144}\text{Nd}$ versus $^{176}\text{Hf}/^{177}\text{Hf}$ for basalts from Oahu compared with other ocean island basalts and MORB^{40,41}. The solid horizontal line represents present-day chondritic Hf and the broken line represents present-day 'bulk Earth' Hf estimated by Patchett and Tatsumoto⁴⁰.

Table 1 Sample locations of Honolulu and Koolau series basalts

Sample no.	Sample	Location
Honolulu series		
OA-1	Mellilite nephelinite	Moliili Quarry, Honolulu, Oahu, (analysed for lead as HMc-2 in ref. 44)
OA-3	Nephelinite	Upper Nuuanu lava flow, Poli Highway west side, 0.07 miles south of Wyllie St, Oahu
OA-4	Nephelinite	Castle lava flow, Kapaa Quarry old face, Kailua, Oahu
OA-5	Nephelinite	Training School lava flow, Poli Highway, ~0.2 miles south of Waimanalo junction, Oahu
OA-6	Alkali-olivine basalt	Kaupo lava flow, highway just east of entrance to Sea Life Park, Oahu
OA-10	Alkali-olivine basalt	Kalama lava flow, highway at east end of Sandy Beach, Oahu
Koolau series		
OA-2	Tholeiite	Koolau Range, Poli Highway east 0.15 miles south of Wyllie St, Oahu
OA-7	Tholeiite	Dyke in roadcut near Makapuu Point, Oahu, (analysed for lead as HMc-1 in ref. 44)
OA-8	Tholeiite	Lava flow in roadcut near Makapuu Point, ~300' E of OA-7, Oahu
OA-9	Tholeiite	Lava flow in roadcut near Makapuu Point, ~700' E of OA-7, Oahu
OA-11	Tholeiite	Lava flow behind no. 6518 Hawaii Kai Drive, Hawaii Kai, Oahu

Koolau and Honolulu series basalts were collected in 1965 by the late G. A. Macdonald and one of us (M.T.). C-series basalts from Waianae have been described by Macdonald and Katsura^{5,6}. Locations for Waianae samples 5X-667-3 and 5X-197 are given by Lanphere *et al.*⁷.

the three groups could be the result of metasomatism of the Honolulu source.

Another important aspect of the data in Table 2 is that in spite of the rather large variations in isotopic compositions among the three series, the shield-building tholeiites and the late-stage alkali basalts of the Waianae volcano have indistinguishable isotopic compositions for all four elements analysed. The results, therefore, point to a common source for the two rock-types.

Nd-Sr, Hf-Sr, Hf-Nd and Sr-Pb relationships among the three series are shown in Figs 1-3. The data fall into three distinct groups on all diagrams. The post-erosional Honolulu basalts plot close to mid-oceanic ridge basalts (MORB) and are characterized by the most radiogenic Hf, Nd and Pb and the least-radiogenic Sr. Koolau tholeiites have the least radiogenic Hf, Nd and Pb and most radiogenic Sr and plot away from MORB towards 'bulk Earth'. Waianae basalts show an intermediate position on all diagrams.

The three groups form a trend on the Nd-Sr diagram (Fig. 1) that is subparallel to the mantle array (solid line) with a steeper negative slope. The Honolulu basalts plot in the upper left portion of the diagram and are at the upper limit for Hawaiian basalts defined by O'Nions *et al.*¹². Koolau tholeiites are unique

Table 2 Nd, Hf, Sr and Pb isotopic compositions* of Oahu volcanics

Sample	Rock type	$^{143}\text{Nd}/^{144}\text{Nd} \pm$	$\epsilon_{\text{Nd}} \pm$	$^{177}\text{Hf}/^{176}\text{Hf} \pm$	$\epsilon_{\text{Hf}} \pm$	$^{87}\text{Sr}/^{86}\text{Sr} \parallel$	$^{206}\text{Pb}/^{204}\text{Pb} \parallel$	$^{207}\text{Pb}/^{204}\text{Pb} \parallel$	$^{208}\text{Pb}/^{204}\text{Pb} \parallel$
Honolulu series									
OA1	Mellilite nephelinite	0.513050 $\pm$ 29	+8.0	0.283224 $\pm$ 29	+12.9	0.70321 $\pm$ 3	18.038 $\pm$ 0.012	15.443 $\pm$ 0.015	37.689 $\pm$ 0.047
OA3	Nephelinite	0.513031 $\pm$ 10	+7.6	0.283204 $\pm$ 53	+12.2	0.70318 $\pm$ 3	18.170 $\pm$ 0.014	15.468 $\pm$ 0.016	37.822 $\pm$ 0.051
OA4	Nephelinite	0.513060 $\pm$ 32	+8.2	0.283319 $\pm$ 65	+16.2	0.70325 $\pm$ 2	18.202 $\pm$ 0.028	15.451 $\pm$ 0.026	37.815 $\pm$ 0.074
OA5	Nephelinite	0.513050 $\pm$ 21	+8.0	0.283279 $\pm$ 51	+14.8	0.70317 $\pm$ 5	18.154 $\pm$ 0.016	15.461 $\pm$ 0.017	37.773 $\pm$ 0.052
OA6	Alkali-olivine basalt	0.513040 $\pm$ 49	+7.8	0.283245 $\pm$ 31	+13.6	0.70328 $\pm$ 4	18.099 $\pm$ 0.018	15.463 $\pm$ 0.019	37.754 $\pm$ 0.055
OA10	Alkali-olivine basalt	0.513049 $\pm$ 10	+8.0	0.283214 $\pm$ 48	+12.5	0.70325 $\pm$ 6	18.103 $\pm$ 0.015	15.438 $\pm$ 0.017	37.686 $\pm$ 0.051
Koolau series									
OA2	Tholeiite	0.512730 $\pm$ 45	+1.8	0.283086 $\pm$ 73	+8.0	0.70400 $\pm$ 3	17.826 $\pm$ 0.014	15.440 $\pm$ 0.016	37.763 $\pm$ 0.049
OA7	Tholeiite	0.512702 $\pm$ 10	+1.2	0.283082 $\pm$ 44	+7.9	0.70403 $\pm$ 2	17.898 $\pm$ 0.015	15.448 $\pm$ 0.017	37.779 $\pm$ 0.052
OA8	Tholeiite	0.512700 $\pm$ 10	+1.2	0.283012 $\pm$ 35	+5.4	0.70411 $\pm$ 3,	18.005 $\pm$ 0.012	15.468 $\pm$ 0.014	37.856 $\pm$ 0.047
							17.994 $\pm$ 0.013	15.456 $\pm$ 0.015	37.828 $\pm$ 0.048
							17.919 $\pm$ 0.014	15.470 $\pm$ 0.016	37.762 $\pm$ 0.050
OA9	Tholeiite	0.512701 $\pm$ 13	+1.2	0.283034 $\pm$ 80	+6.2	0.70412 $\pm$ 3	17.929 $\pm$ 0.018	15.484 $\pm$ 0.019	37.803 $\pm$ 0.055
							17.933 $\pm$ 0.013	15.476 $\pm$ 0.016	37.783 $\pm$ 0.050
OA11	Tholeiite	0.512698 $\pm$ 17	+1.1	0.283041 $\pm$ 18	+6.4	0.70403 $\pm$ 3	17.912 $\pm$ 0.026	15.445 $\pm$ 0.025	37.749 $\pm$ 0.070
Waianae series									
5X-667-3	Tholeiite	0.512940 $\pm$ 21	+5.9	0.283153 $\pm$ 29	+10.4	0.70362 $\pm$ 6	—#	—	—
C46	Tholeiite	0.513005 $\pm$ 16	+7.1	0.283120 $\pm$ 50	+9.2	0.70351 $\pm$ 2	18.158 $\pm$ 0.012	15.449 $\pm$ 0.015	37.762 $\pm$ 0.050
C48	Tholeiite	0.512974 $\pm$ 26	+6.5	0.283126 $\pm$ 29	+9.4	0.70357 $\pm$ 3	18.114 $\pm$ 0.016	15.454 $\pm$ 0.018	37.735 $\pm$ 0.054
5X-197	Alkali basalt	0.512950 $\pm$ 25	+6.0	0.283225 $\pm$ 36	+12.9	0.70362 $\pm$ 5	—#	—	—
C30	Alkali basalt	0.512959 $\pm$ 22	+6.2	0.283134 $\pm$ 32	+9.7	0.70366 $\pm$ 3	18.143 $\pm$ 0.014	15.457 $\pm$ 0.016	37.754 $\pm$ 0.050
C52	Hawaiite	0.512971 $\pm$ 15	+6.5	0.283162 $\pm$ 38	+10.7	0.70357 $\pm$ 3	18.082 $\pm$ 0.013	15.439 $\pm$ 0.015	37.692 $\pm$ 0.048

* Uncertainties are 2σ mean.† Normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$. Adjusted for instrumental bias to $^{143}\text{Nd}/^{144}\text{Nd} = 0.511860$ for the La Jolla Nd standard (BCR-1 = 0.51262 ± 1).‡ ϵ s are deviations in parts per 10^4 from present-day chondritic Nd (0.51264) and Hf (0.28286).§ Normalized to $^{179}\text{Hf}/^{177}\text{Hf} = 0.7325$. A mean $^{176}\text{Hf}/^{177}\text{Hf} = 0.282211 \pm 13$ was obtained for Hf standard JMC475.|| Normalized to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$; adjusted for instrumental bias to $^{87}\text{Sr}/^{86}\text{Sr} = 0.71014$ for NBS standard SRM-987.¶ Corrected for blank and mass fractionation of $0.12 \pm 0.03\%$ per AMU based on analyses of NBS standard SRM-982. ^{206}Pb blank/sample ≤ 0.0025 .

Samples were received as powder splits and were not analysed for Pb.

among Hawaiian basalts thus far analysed and plot close to 'bulk Earth'.

Sediment or seawater contamination will generally produce a shift to the right of the mantle array in both the Nd–Sr and Hf–Sr diagrams (Figs 1 and 2a). Thus, a small amount of sediment contamination might account for the slight shift of the Waianae and Honolulu data to the right of the mantle array in the Nd–Sr diagram. However, the Hf–Sr relationships show no evidence for radiogenic Sr addition. In any case, the differences in isotopic compositions among the three groups and the position of Koolau data below the mantle array are probably not due to sediment contamination, nor can the differences be easily attributed to mixing between ancient sediment and a MORB-type source¹³.

The Sr–Pb diagram (Fig. 3) provides further evidence that sediment or seawater contamination did not have an important role in generating the isotopic characteristics of the three series. A positive correlation between $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$, either within a suite or between suites, can be indicative of sediment contamination (but, of course, does not require contamination). No such correlation is observed among the three groups or within the Waianae and Honolulu basalts. An apparent weak positive correlation is observed among Koolau tholeiites which could be indicative of contamination. However, contamination by sediment with typical seawater Pb and Sr isotopic compositions ($^{87}\text{Sr}/^{86}\text{Sr} \sim 0.708$, $^{206}\text{Pb}/^{204}\text{Pb} \sim 18.9$) should produce a much steeper slope than that exhibited by the Koolau samples (see Fig. 3). If Koolau basalts are slightly contaminated, then uncontaminated Koolau tholeiites would plot even further below the mantle trend on the Sr–Nd diagram.

If we include Pb isotopic data from the island of Hawaii¹⁴ combined with preliminary Sr isotopic data from the same samples¹⁵, then there is evidence for yet another trace-element source for Hawaiian basalts with radiogenic Pb ($^{206}\text{Pb}/^{204}\text{Pb} \geq 18.5$) and a Sr isotopic composition similar to those of Waianae basalts (arrow in Fig. 3).

Pb isotopic compositions are plotted on $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagrams in Fig. 4. Also shown are Pb isotopic data from the island of Hawaii¹⁴ and the mean oceanic trend defined primarily by MORB but including many oceanic islands and seamounts^{16–18}. The

Hawaiian Loa and Kea trends are defined by Jackson *et al.*¹⁹ and have distinct Pb isotopic compositions¹⁴ with basalts from the Kea group (Kohala, Mauna Kea, Kilauea) having more radiogenic Pb than basalts from the Loa Group (Hualalai, Mauna Loa). According to Jackson *et al.*, Koolau and Waianae belong to the Loa Group.

Pb data from Honolulu and Waianae basalts plot on the Hawaii trend in Fig. 4b in the vicinity of other Loa samples. However, in Fig. 4a these basalts plot on an extension of the Kea trend. Thus, while the Waianae and Honolulu sources appear to have evolved with a U/Pb ratio characteristic of other Loa samples, the Th/U ratio in this source was lower than that in the source for Loa basalts on Hawaii.

Koolau samples show the least radiogenic Pb (in terms of $^{206}\text{Pb}/^{204}\text{Pb}$) of any Pacific oceanic basalts and plot on or slightly above the Hawaiian trend. The Koolau data are also unique on the $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagram (Fig. 4a). The data plot to the left of the oceanic trend and seem to indicate a higher Th/U ratio in the Koolau source than is common in oceanic basalt sources. The apparent higher Th/U ratio in the Koolau source is consistent with its less-depleted character (relative to other Oahu volcanics) inferred from Sr, Nd and Hf relationships. The ^{208}Pb – ^{206}Pb relationships in Fig. 4a also require at least three sources: a high U/Pb source for basalts of the Kea trend on Hawaii, a low U/Pb and low Th/U source for Honolulu and Waianae basalts, and a low U/Pb but high Th/U source for Koolau tholeiites.

The linear trend defined by the Hawaii data in Fig. 4b corresponds to an apparent age of 940 Myr (ref. 14). This apparent age is younger than the 1,760 Myr apparent age of oceanic basalts in general¹⁴, and the 1,500–1,800 Myr apparent ages of MORB and other individual oceanic islands^{16–18,20}. The 940-Myr apparent age for Hawaii will have true significance only if the various sources of Hawaiian basalts formed at the same time from a single reservoir.

The interpretation of the apparent ^{207}Pb – ^{206}Pb age depends heavily on whether the two Loa and Kea trends are isotopically distinct. If, as we currently believe¹⁵, the two trends are distinct, then either two 'mantle plumes'²¹ or two melting zones at or below the base of the lithosphere are required. In the latter case, the Loa and Kea trends would reflect different proportions

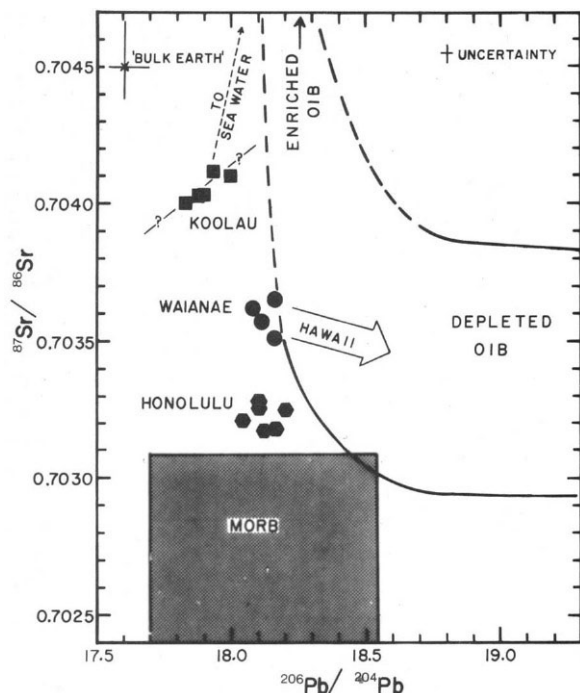


Fig. 3 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ for basalts from Oahu. Shown for comparison are fields for MORB and oceanic island basalts (OIB); for a summary see Zindler *et al.*²⁶. Bulk-Earth Sr is from Fig. 2 and bulk-Earth $^{206}\text{Pb}/^{204}\text{Pb}$ (~ 17.6) represents the mean intersection of the mantle trend (Fig. 4) with the geochron. An apparent weak correlation among Koolau tholeiites is indicated by the queried broken line and a seawater contamination trend by a steeper broken line. The Hawaii arrow represents the field of preliminary data from this laboratory^{14,15}.

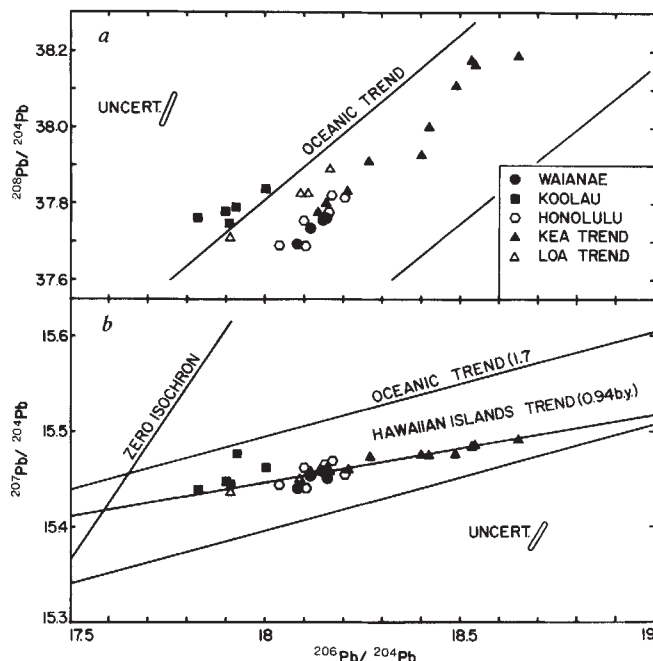


Fig. 4 *a*, $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagram for basalts from Oahu and Hawaii^{13,14}. Koolau data plot to the left of the mantle trend^{14,17-19} whereas Waianae, Honolulu and Hawaii data plot within the trend. *b*, $^{207}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ diagram of basalts from Oahu. The mantle trend^{14,17-19} for oceanic basalts corresponds to an apparent 1,700 Myr age whereas data from Hawaii¹⁴ corresponds to an apparent 940 Myr age. Oahu data plot on or slightly above the Hawaii trend.

of plume, lithosphere and asthenosphere in the source magmas and the apparent age would almost certainly have no significance. On the other hand, if future work shows that isotopic variations are random with respect to the Loa and Kea loci, then arguments for a single isotopically heterogeneous source become more reasonable²⁰. In either case, the slight deviation of the Koolau data from the Hawaiian trend in the ^{207}Pb – ^{206}Pb plot (Fig. 4*b*) suggests that at least some Hawaiian basalts were derived from sources unrelated in age to one another.

Relationships among Oahu volcanic rocks

Three isotopically distinct sources for Oahu volcanics appear to have existed; a strongly-depleted source for Honolulu alkalic–nephelinitic rocks, a slightly-depleted source for Koolau tholeiites, and an intermediate source for Waianae volcanics. However, in spite of this isotopic evidence for multiple sources, the isotopic compositions of the shield-building tholeiites and the late-stage alkali basalts of the Waianae volcano are indistinguishable. The Waianae data support Tatsumoto's¹⁴ conclusion, based on Pb isotopes in Hawaiian volcanics, that there is a close genetic relationship between the two rock types, but Sr isotopic data of Lanphere *et al.*⁷ for Hawaiian–Emperor volcanics suggest that this may not be true for all volcanoes in the chain.

In contrast, the isotopic data of Koolau and Honolulu suggest independent origins for some tholeiites and spatially associated alkali basalt–nephelinite suites^{22–24}. Thus, at least on Oahu, two types of alkalic rocks exist: one whose source is the same as that of the shield-building tholeiites and another, more nephelinitic suite, whose source is isotopically distinct.

Considering the time frame for the sequence of eruption on Oahu^{2,3,19}, and assuming that the Hawaiian island-chain reflects movement of the lithosphere over a fixed hotspot²⁵ or plume²¹

in the lower mantle, then a different source for Honolulu volcanic rocks may be expected. Waianae and Koolau were apparently formed as a direct result of the mantle plume. However, when the Honolulu volcanics were erupting the Pacific plate had moved about 260 km to the north-west and the plume was forming the Kohala and Hualalai volcanoes on the island of Hawaii. Thus, the Honolulu series seems to have no relationship to the plume and appears to have been generated by some other mechanism such as elastic unloading²².

Unfortunately, a complete eruptive sequence does not exist at either volcano on Oahu, so we are not able to compare the isotopic characteristics of caldera-filling and post-erosional volcanics on a single volcano. Furthermore, even shield-building tholeiites of the adjacent Waianae and Koolau volcanoes appear to have been derived from different sources. Consequently, the isotopic relationships between post-erosional volcanics and the main- and late-stage basalts may be different for other volcanoes.

If the Waianae and Koolau volcanoes originated from the same mantle plume, the differences in isotopic compositions between these two volcanoes may represent different degrees of assimilation of upper mantle material into the mantle plume¹⁴ or that the upper mantle is isotopically heterogeneous. The oceanic lithosphere would initially have MORB-type Sr, Pb, Nd and Hf isotopic compositions, but, depending on the age of the lithosphere, it may have much more radiogenic Pb than present-day MORB (although among present-day Pacific MORB there is a wide range in $^{206}\text{Pb}/^{204}\text{Pb}$ ratios). This is because the half life of ^{238}U is much shorter than those of ^{87}Rb , ^{147}Sm and ^{176}Lu so that recent ($\sim 10^8$ yr) parent–daughter fractionation produces a much larger effect on the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio than on the isotopic compositions of the other daughter elements. Therefore, Tatsumoto¹⁴ proposed that the radiogenic Pb found in the Kea volcanics on Hawaii may reflect

contamination or assimilation of ~100-Myr old lithosphere. If this is so, then it is rather difficult to ascribe the significant differences in Sr, Nd and Hf isotopic compositions between Waianae and Koolau, yet rather small differences in Pb isotopic compositions to contamination or assimilation of the same lithosphere that produced such radiogenic Pb on Hawaii. Thus, we conclude that the two tholeiitic suites on Oahu have derived their trace elements from isotopically distinct deep-mantle may reflect either another mantle source or a metasomatic component¹⁰.

Recently, Zindler *et al.*²⁶ have proposed that the Pb, Sr and Nd isotopic characteristics of oceanic basalts in general, can mathematically be described as mixtures of exactly three components. That is, when Sr, Nd and Pb isotopic compositions are plotted in three dimensions, oceanic basalt data lie on a single mantle plane. Waianae and Honolulu data plot precisely on the mantle plane. The location of Honolulu data on the plane is rather surprising if, in fact, the source region of these basalts was metasomatized^{10,26}. On the other hand, Koolau data plot significantly below the mantle plane. If the deviation of Koolau data is ascribed to one isotope system, then either ¹⁴³Nd/¹⁴⁴Nd is 0.032% too low, ⁸⁷Sr/⁸⁶Sr is 0.104% too low, or ²⁰⁶Pb/²⁰⁴Pb is 16% too low. The deviation of Koolau from the mantle plane is more than twice that of any of the data used by Zindler *et al.* to construct the mantle plane. If, as previously mentioned, Koolau volcanics have suffered slight sediment contamination, then data from uncontaminated Koolau samples would plot even further below the plane. The fact that Koolau Sr, Nd, Hf and Pb isotopic compositions are close to presumed 'bulk Earth' values, yet do not plot on the mantle plane, raises a serious question regarding the significance of the three endmembers that comprise the plane. White *et al.*²⁷ have shown that data from Samoa and the Society Islands plot distinctly above the mantle plane, and we concur with these authors that the mantle must be (mathematically) composed of at least four components. Of greater importance is the fact that the apparent coherent fractionation of Rb-Sr, Sm-Nd and U-Pb implied by the mantle plane may be invalidated by the more recent data. Either the isotopic characteristics of oceanic basalts are governed by more than three factors (including both chemical fractionation and ages) or U/Pb fractionation occurs independently from Rb/Sr, Sm/Nd, and Lu/Hf fractionation^{28,29}. The apparent decoupling of U-Pb fractionation from that of other isotope systems may be due to the comparatively large geochemical mobility of Pb and reflect, in part, transfer of Pb to the core³⁰ or secondary effects such as contamination from the lithosphere¹³, sediments, or ancient recycled oceanic crust¹². However, the data may also, in part, reflect recent (~10⁸-10⁹ yr) secondary parent-daughter fractionation events in the mantle sources. Due primarily to the comparatively short

half life of ²³⁸U, these recent events produce appreciable change only in the Pb isotopic composition (²⁰⁶Pb/²⁰⁴Pb).

Conclusions

Volcanic rocks on Oahu appear to have been derived from three isotopically distinct sources: (1) the post-erosional Honolulu volcanics were derived from a highly-depleted (MORB-like) source; (2) shield-building tholeiites of the Koolau volcano were derived from a very-slightly-depleted source, and (3) shield-building tholeiites and caldera-filling alkali basalts of the Waianae volcano were derived from a source with Sr, Nd and Hf isotopic characteristics intermediate between Koolau and Honolulu, but with Pb isotopic compositions indistinguishable from those of Honolulu series volcanics.

A fourth source (lithosphere?) for trace elements in Hawaiian basalts in general is implied by the radiogenic Pb isotopic compositions on the island of Hawaii. Thus the 940-Myr apparent Pb-Pb age for the Hawaiian islands apparently has no rigorous significance.

Alkalic rocks on Oahu were derived from two different sources. Caldera-filling alkali basalts on the Waianae volcano were apparently derived from the same source as the shield-building tholeiites of this volcano. Post-erosional Honolulu series alkali olivine basalts and nephelinites were derived from a source with no apparent genetic relationship to the tholeiitic source of the Koolau volcano, despite an intimate spatial relationship between the Koolau and Honolulu series.

Sr, Nd and Pb relationships for Koolau basalts suggest that their source may be unique, not only among Hawaiian basalts, but among oceanic basalts in general. Koolau data plot below the mantle plane and reaffirm older theories that Rb/Sr, Sm/Nd and U/Pb fractionations are not necessarily coherent. The near 'bulk-Earth' Sr, Nd and Hf isotopic compositions of Koolau tholeiites, and their rather non-radiogenic Pb isotopic compositions which plot closer to the Pb-Pb geochron than do any other Pacific basalts yet analysed may suggest that this source region has experienced less net fractionation throughout geological time than have typical oceanic basalt sources. In this context, the importance of the deviation of Koolau data from the mantle plane is magnified.

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A cell-surface molecule involved in organ-specific homing of lymphocytes

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Lymphocytes migrate from the bloodstream by recognizing and binding to specialized endothelial cells lining the high endothelial venules (HEV) in lymph nodes and Peyer's patches. We describe here a monoclonal antibody, MEL-14, specific for a lymphocyte surface molecule that appears to mediate recognition of lymph node HEV, and to be required for lymphocyte homing into lymph nodes in vivo.

THE involvement of specific cell-surface molecules in the selective association of cells has become a central tenet of modern cell biology.^{1,2} Here we have studied a specific cell-cell recognition system involved in the migration of lymphocytes from the blood. In this system lymphocytes bind selectively to specialized vascular endothelial cells in lymphoid organs. We report the identification of one of the cell-surface recognition elements involved.

Most mature lymphocytes are mobile cells, recirculating continuously between the lymphoid organs via both the blood and lymph vasculature³⁻⁵. The migration of lymphocytes from the blood into these lymphoid organs; the spleen, lymph nodes and the gut-associated Peyer's patches, is not random but rather is a process which maintains their architecture and specific cellular composition. Entrance of lymphocytes into lymph nodes and Peyer's patches is mediated by specific interactions between lymphocytes and the endothelial cells lining post-capillary high endothelial venules (HEV)^{6,7}. Within minutes following their release into the bloodstream lymphocytes adhere to these specialized venules, then migrate into the parenchyma of the lymphoid organ. Adherence of lymphocytes to HEV can be examined both in studies of the short-term localization of transfused lymphocyte populations *in vivo*, and in an assay of lymphocyte binding to HEV in frozen sections of murine lymph nodes or Peyer's patches *in vitro*⁸⁻¹². Using these assays, we have demonstrated that mature lymphocytes bind well to HEV while their immature precursors bind very poorly¹³, and at least some of their antigen-stimulated progeny^{11,12} fail to bind at all. This regulation involves not only control of the overall ability to bind to HEV, but also of the specificity of binding to HEV in particular organs. For example, the two major functional subsets of lymphocytes, T cells and B cells, exhibit differential preferences for migrating to peripheral lymph nodes versus gut-associated lymphoid tissues *in vivo*; these differences are determined by preferential adherence of B cells to Peyer's patch HEV, and of T cells to peripheral node HEV¹⁰. Moreover, certain murine lymphomas bind with nearly absolute specificity to Peyer's patch HEV, while others bind almost exclusively to peripheral node HEV (Table 1)¹³. On the basis of these data we have proposed that peripheral node HEV and Peyer's patch HEV bear distinct determinants for lymphocyte recognition, and that most normal lymphocytes express variable levels of surface receptors complementary to each of these endothelial determinants. Tumour cells of restricted specificity would, by this model, bear receptors for only one HEV type.

To study the molecular basis of these interactions, we have generated monoclonal antibodies against a peripheral node HEV-binding lymphoma, 38C-13. By screening for antibodies which distinguish peripheral node HEV-binding lymphoid cells from non-binding or Peyer's patch HEV-specific lymphocytes we isolated a monoclonal antibody, MEL-14, which binds to determinants on or close to the lymphocyte surface molecule mediating lymphocyte recognition of peripheral node HEV.

Production of monoclonal antibody, MEL-14

As we had previously demonstrated a significant difference in HEV binding of mouse and rat cells¹³, a cloned C3H/eb mouse B-cell lymphoma, 38C-13^{14,15}, which binds to peripheral node but not to Peyer's patch HEV was used to immunize Fisher rats for the production of hybridomas as described in Table 1. Hybrid clones were tested by immunofluorescence for antibodies directed against two *in vitro* cell lines, RAW112 and EL4 (which bind neither Peyer's patch nor peripheral node HEV), against normal C57BL/6J mesenteric node lymphocytes (which bind both HEV types), and against the immunogen, 38C-13. Clones whose supernatant antibodies reacted with both 38C-13 and normal lymphocytes but not with RAW112 or EL4 were subcloned by limiting dilution, and supernatants from these subclones were then analysed by a fluorescence activated cell sorter (FACS) against a more extensive panel of cells.

Antibodies from one of these subcloned hybrids, MEL-14, showed a pattern of reactivity which correlated with the ability of cells to adhere to peripheral node HEV (Table 1). None of 10 HEV non-binding lymphoid cell lines had detectable amounts of the MEL-14 antigen on their surface. In addition, two Peyer's patch HEV-specific neoplasms (a T-cell lymphoma and the mastocytoma, P815) lacked the marker. MEL-14 antibodies also failed to stain cloned, normal T cells maintained *in vitro* and another group of cells which do not adhere to HEV or migrate normally *in vivo*¹². All four of the peripheral lymph node HEV-specific lymphomas and one lymphoma with dual HEV-binding specificity were MEL-14 antigen positive, as were the majority of normal lymph node lymphocytes from the four strains tested. MEL-14 antibody staining did not correlate with H-2 type, cell class, strain of origin, or with the expression of known lymphocyte differentiation antigens.

As noted above, in comparison with mature lymphocytes, cells from bone marrow and thymus bind poorly to HEV^{9,13}, as do B-cell blasts from germinal centres¹¹. The intensity of MEL-14 staining of pre-B cells and of most thymocytes was approximately 10-fold less than on mature lymphocytes (Table 1b). Germinal centre blasts¹¹ and Abelson pre-B lymphomas were not stained by MEL-14.

MEL-14 blocks lymphoma cell adherence

The data presented in Table 1 are consistent with the possibility that MEL-14 detects a determinant present on or associated with a receptor for peripheral lymph node HEV. We therefore tested whether MEL-14 antibodies would interfere with the interaction of lymphoid cells with peripheral node HEV *in vitro*. 38C-13 cells were pre-coated with saturating levels of either MEL-14 or various control antibodies, as described in the legend to Fig. 1. To test their HEV-binding activity the antibody-coated lymphoma cells were then incubated on frozen sections of mesenteric lymph nodes as described previously⁹.

Table 1 Correlation of HEV-binding phenotype and expression of the MEL-14 antigen

Cell	Type	Binding to HEV (RAR $\pm$ s.e)		Expression of MEL-14 antigen (Modal fluorescence)
		Peripheral lymph node	Peyer's patches	
<i>a</i> Tumours				
L1-2	Pre-B cell	0*	0	0
RAW112	Pre-B cell	0	0	0
p90/m	Pre-B cell	0	0	0
p120/AKV	Pre-B cell	0	0	0
p160/m	Pre-B cell	0	0	0
T69	B cell	0	0	0
EL4	T cell	0	0	0
KKT2	T cell	0	0	0
TK52	T cell	0	0	0
TK54	T cell	0	0	0
TK50	T cell	0.07 $\pm$ 0.03	0.91 $\pm$ 0.19	0
P815	Mastocytoma	0	0.23 $\pm$ 0.09	0
38C-13	B cell	1.1 $\pm$ 0.1	0	196
BK37	B cell	1.7 $\pm$ 0.2	0	80
MBL-2	T cell	0.23 $\pm$ 0.09	0	167
UNC-1	T cell	0.15 $\pm$ 0.07	0	92
TK38	T cell	0.60 $\pm$ 0.11	0.36 $\pm$ 0.06	113
<i>b</i> Normal cells				
Mesenteric node lymphocytes		Unity	Unity	100
Pre-B cells				8
Thymocytes		0.05 $\pm$ 0.01		10
T-cell clones		0	0	0

Monoclonal antibodies: An initial footpad injection of 5×10^7 38C-13 cells in complete Freund's adjuvant was followed in 15 days by a second footpad immunization with 5×10^7 cells in phosphate-buffered saline. Three days later the immunized rat's spleen cells were fused³⁹ with the p3/ $\times$ 63/Ag8.653 nonproducer mouse myeloma line⁴⁰ and selected in hypoxanthine-aminopterin-thymidine medium. Binding to HEV: The ability of lymphoid cells to adhere to high endothelial venules (HEV) was determined as described previously⁹. Normal lymphocyte populations and lymphoid tissues for sections were usually from 4-8 week old (C57BL/6J $\times$ BALB/c)F₁ hybrids. To allow quantitative comparisons, an internal standard population of fluorescent mesenteric node lymphocytes, labelled by 15 min incubation with 75 μ g ml⁻¹ fluorescein isothiocyanate (FITC) at 37 °C, was mixed with each test population before incubation^{9,16,17}. The ratios of sample to standard cells in the incubation mixtures (R₁) and adherent to venules (R_{HEV}) were determined and the specific adherence ratio, R_{HEV}/R₁, was calculated for each sample (SAR_s) and for normal mesenteric node lymphocytes (SAR_m). Direct comparison of the adherence capacity of sample cells to that of normal unlabelled mouse mesenteric node lymphocytes is given as a relative adherence ratio (RAR_s) = SAR_s/SAR_m (with the RAR of normal lymphocytes = unity). Standard errors of all ratios were calculated by the delta method⁴¹. Cells: The tumour cell lines used in these studies are as follows: L1-2, an Abelson virus-induced C57L lymphoma and RAW112⁴², an Abelson virus-induced BALB/c lymphoma (from R. Coffman, DNAX Corp.); p90/m, p120/AKV, and p160/m, Abelson virus-induced C57L lymphomas, (From C. Whitlock and O. Witte, UCLA); T69, a B10.A.5R lymphoma (from J. Monaco, Stanford University); EL4, a chemical carcinogen-induced C57BL lymphoma; KKT2⁴⁰, TK38, TK50, TK52, and TK54, spontaneous AKR lymphomas; P815, a chemical carcinogen-induced, DBA/2 mastocytoma; 38C-13^{4,15}, a chemical carcinogen-induced C3H/eb lymphoma; MBL-2, a Moloney leukaemia virus-induced C57BL lymphoma; UNC-1, a B10.H-2^a H-4^bp/wts lymphoma (a gift of G. Houghton, University of N. Carolina); and BK37, an AKR/Cum lymphoma. This table includes a summary of results obtained from five independently derived T-cell clones of both helper and cytolytic phenotype. Clones were derived as described previously (refs 12, 43 and M. Dailey, unpublished). FACS analysis: 10⁶ viable, nucleated cells were incubated on ice for 30 min in 10 μ l of phosphate buffered saline with 1mM sodium azide containing at least 1 μ g m of MEL-14 antibody (a saturating level) or negative control rat monoclonal antibody, washed, and incubated with fluoresceinated rabbit anti-rat immunoglobulin antibodies (rendered specific for rat immunoglobulin by sequential negative and positive selection on mouse and rat immunoglobulin columns) for 7-10 min on ice. All reagents were used at saturating levels. Following thorough washing the cells were analyzed on a Becton Dickinson FACS III, described previously¹⁷. The modal fluorescence obtained with MEL-14 is expressed here after subtraction of modal fluorescence obtained with negative control antibodies. The modal fluorescence of MEL-14 stained normal mesenteric node lymphocytes of all mouse strains tested (A/J, BALB/c, BALB.K, C57BL/6J) was the same, and is here defined as 100 fluorescence units. For comparison, the modal fluorescence of unstained glutaraldehyde fixed chicken red blood cells on this scale is about 400. Bone marrow pre-B cells were identified in two-colour analysis on a modified FACS-II with the monoclonal antibody, RA3-6B2, which is specific for the B cell form of the T200 family of molecules (B220)⁴⁴. The RAR value for selected populations of pre-B cells from bone marrow was not directly assayed. The RAR on peripheral lymph node HEV for unfractionated, nucleated bone marrow cells (0.06 $\pm$ 0.02) may reflect the number of mature lymphocytes present in the population⁹.

* 0; RAR < 0.05

While no significant inhibition of binding was evident with any of the control antibodies, pretreatment with MEL-14 almost totally inhibited attachment of 38C-13 cells to the venules without causing significant cell death or agglutination (Fig. 1). The absence of inhibition by anti-T200 (30G12) and anti-idiotypic (4G10) antibodies controls for nonspecific blockade due to Fc receptor effects, since these antibodies are of the same isotype as MEL-14 (IgG2a). To further investigate the specificity of MEL-14 blocking of HEV adherence, we tested the ability of MEL-14 to inhibit the binding of normal lymphocytes to peripheral node and Peyer's patch HEV.

MEL-14 blocking of normal cell binding

Because MEL-14 does not stain Peyer's patch HEV-specific tumours (Table 1), we postulated that its inhibitory effect would

be organ-specific. To test this, normal mesenteric node lymphocytes, which bind to both HEV types, were pretreated as above with MEL-14 and incubated on sections of peripheral lymph nodes and Peyer's patches. (Again, no evidence for cytotoxicity or agglutination was found.) Pretreatment with MEL-14 almost completely blocked the binding of normal lymphocytes to peripheral node HEV ($P < .0001$), without significantly altering their binding to Peyer's patch HEV (Fig. 2).

The interpretation that this inhibition results from specific blockade of a receptor for peripheral node HEV determinants (and not from membrane alterations or other nonspecific effects induced by bound antibody) assumes that individual lymphocytes express receptors for both HEV types. Alternatively, independent lymphocyte subpopulations might exist which bind exclusively to either peripheral lymph node or Peyer's patch

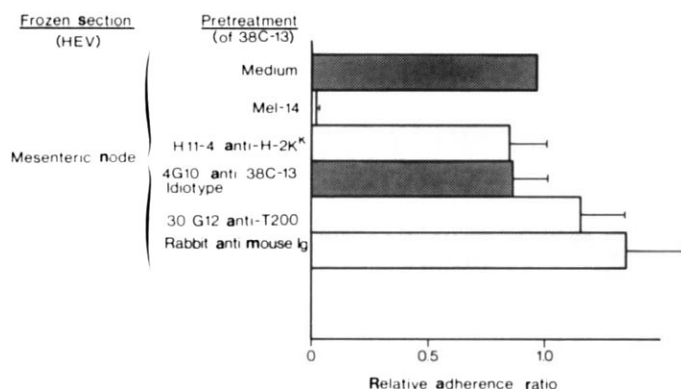


Fig. 1 Preincubation of 38C-13 cells with MEL-14 but not with other antibodies inhibits their ability to adhere to HEV. 10^7 38C-13 cells were incubated on ice for 1 h with medium or with saturating amounts of one of the following antibodies: MEL-14; H11-4⁴⁵, mouse monoclonal anti-H-2K^k (biotinylated); 4G-10⁴⁶, rat monoclonal anti-38C-13 idiotype (a gift from D. Maloney); 30G-12⁴⁷, rat monoclonal anti-T200; and polyclonal, affinity purified rabbit anti-mouse immunoglobulin. The relative amounts of antibody bound in each case were assessed by FACS analysis of an aliquot of sample cells stained with an appropriate second stage reagent; either fluoresceinated avidin (Vector Labs, San Mateo) rabbit anti-rat immunoglobulin or goat anti-rabbit immunoglobulin. The modal fluorescence intensities (expressed in arbitrary fluorescence units with MEL-14 staining of normal mesenteric node lymphocytes equal to 100 units) were as follows: MEL-14, 256 units; H11-4, 204 units; 4G-10, 228 units; 30G-12, 301 units; rabbit anti-mouse Ig, 161 units. After thorough washing, test cells were checked for agglutination and for viability by Trypan blue exclusion and by FACS analysis of forward light scatter⁴⁸. In each case less than 5% of the cells were found to be either dead or agglutinated. Test cells were then incubated on fresh frozen sections of (C57BL/6 × BALB/c)F₁ mesenteric lymph nodes. Assay conditions were the same as described for Table 1 except that the internal standard cells were FITC-labelled, untreated 38C-13 cells.

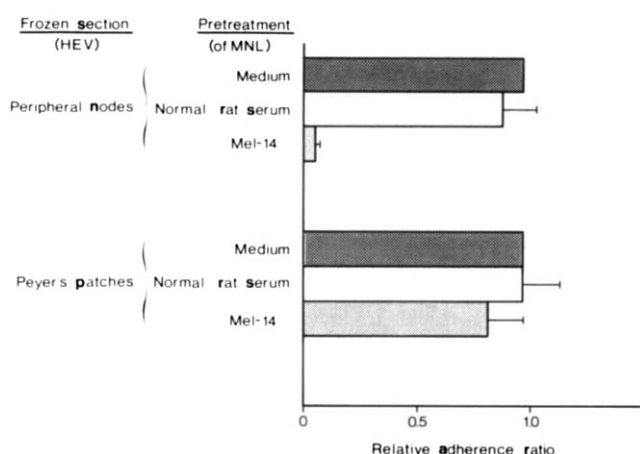


Fig. 2 Pretreatment with MEL-14 blocks the binding of normal lymphocytes to peripheral node HEV but not to Peyer's patch HEV. 10^7 (C57BL/6 × BALB/c)F₁ mesenteric node lymphocytes were pretreated with medium alone, with normal rat serum, or with a saturating amount of MEL-14 antibody as described in Fig. 1. After washing, cells were mixed with an internal standard population of untreated FITC-labelled normal lymphocytes and incubated as before on frozen sections of peripheral lymph nodes and Peyer's patches. Data were collected and reduced as described in Table 1. The fluorescent internal standard population was omitted in a parallel experiment in which the sections and HEV-adherent cells were stained with FITC-rabbit anti-rat immunoglobulin to determine the proportion of Peyer's patch HEV-bound cells bearing MEL-14 antibodies (see text).

HEV. The possibility that Peyer's patch HEV-binding cells might be MEL-14 negative was tested by preincubating mesenteric node lymphocytes with MEL-14, allowing them to adhere *in vitro* to HEV in Peyer's patches, and then counterstaining them with fluoresceinated rabbit anti-rat immunoglobulin. Even though MEL-14 effectively inhibited binding to peripheral node HEV in this experiment, most cells adhering to Peyer's patch HEV were indeed MEL-14 positive ($88 \pm 3\%$ MEL-14 positive compared with $2 \pm 1\%$ positive observed with an isotype matched negative control, 11B-1; see Fig. 4 legend). Thus MEL-14 specifically blocks the binding of MEL-14 antigen positive cells to lymph node HEV, without blocking binding of MEL-14 antigen positive cells to Peyer's patch HEV. This selectivity makes it unlikely that MEL-14 pretreatment causes nonspecific alteration of overall adhesive capacity and argues that MEL-14 binds to a determinant on, or in intimate association with, molecules mediating recognition of lymph node HEV.

MEL-14 blocks lymphocyte homing *in vivo*

To test whether the structure(s) recognized by MEL-14 were also required for migration of lymphoid cells in physiological conditions *in vivo*, we directly labelled normal mesenteric node lymphocytes with tetramethylrhodamine isothiocyanate as described^{16,17}, pretreated them as above with either medium alone, MEL-14, or 30G12 (anti-T200) and injected them intravenously with an equal number of fluorescein isothiocyanate (FITC)-labelled¹⁷ untreated lymphocytes into syngeneic recipients. After 30 min the relative numbers of test (red) and internal standard (green) cells in cell suspensions of the recipients' lymph nodes and Peyer's patches were determined by dual immunofluorescence microscopy. The results (Fig. 3) were similar to those observed *in vitro*. MEL-14 markedly ($P < .0001$ in both experiments) inhibited the migration of pretreated cells into peripheral nodes. Only a slight inhibition of migration into Peyer's patches was found, even though restaining with MEL-14 revealed that 77% of the pretreated cells that localized in Peyer's patches were MEL-14 antigen positive. (In fact, 52% still bore residual MEL-14 antibody, detectable with fluoresceinated rabbit anti-rat Ig alone; see Fig. 3 legend.) The modest reduction in Peyer's patch localization was probably a non-specific effect, possibly Fc mediated, as similar results were obtained when the sample population was pretreated with the isotype-matched control 30G12. In contrast to MEL-14 treated cells, however, 30G12 coated cells localized equally well (relative to untreated cells) in both lymph nodes and Peyer's patches. These results indicate that MEL-14 binds to surface structures specifically required for lymphocyte migration into lymph nodes *in vivo*.

MEL-14 binds to one molecule class

Following radioiodination of lymphocytes, the cell-surface molecules bound by MEL-14 were analysed by SDS-polyacrylamide gel electrophoresis (PAGE, Figs 4, 5). In reducing conditions, a single band with an apparent molecular weight of 80,000 (80 K) was detected in lysates of normal mesenteric node lymphocytes (Fig. 4b), and a single 92 K component was identified in lysates of 38C-13 cells (Fig. 4c). These bands were absent from samples precipitated with an isotype matched (IgG2a) negative control antibody (11B-1, Fig. 4a, d). The 80 K band was characteristic of normal mesenteric node lymphocytes from several other strains of mice as well (A/J, C57BL/Ka, BALB/c, BALB.K and C57BL/6; data not shown).

The apparent size difference between the molecules identified on normal lymphocytes and 38C-13 cells may be due to differing patterns of glycosylation, but more detailed biochemical analyses will be necessary to clarify this point. Surprisingly, in nonreducing conditions the mobility of the immunoprecipitated molecules increased, resulting in a slight decrease in the estimate of the apparent molecular weight of both the 80 K normal lymphocyte (Fig. 5c, d), and the 38C-13 derived 92 K species. The absence of higher molecular weight forms in nonreducing

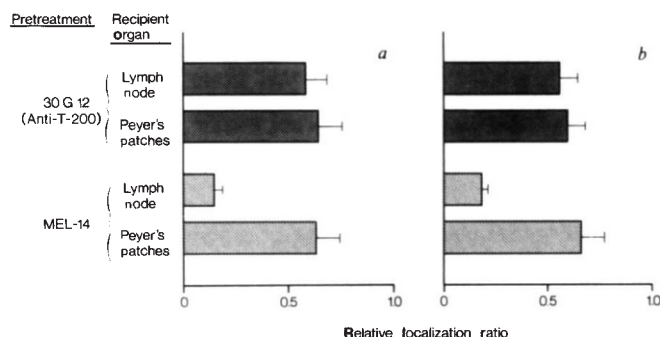


Fig. 3 MEL-14 pretreatment selectively inhibits the homing of lymphocytes into lymph nodes *in vivo*. 5×10^7 TRITC-labelled mesenteric node lymphocytes from 4–8 week old AKR/Cum (a) or BALB/c (b) mice were pretreated with saturating levels of MEL-14 antibody (100 μ g/ml, affinity purified on a rabbit anti-rat immunoglobulin column) or of 30G-12 (anti-T200) as described in Fig. 2 legend. The antibody-coated cells were washed, mixed with an internal standard population of fluoresceinated syngeneic mesenteric node lymphocytes, and injected intravenously in the tail vein of syngeneic recipients (10^8 total cells per mouse). After 30 min the mice were killed and single cell suspensions of peripheral (axillary, brachial and cervical) lymph nodes and Peyer's patches were prepared. The relative numbers of antibody pretreated and internal standard cells localized in each organ were determined by fluorescence microscopy. A relative localization ratio (RLR), similar to the RAR described in Table 1, was calculated as described previously¹⁷. The RLR is proportional to the % of injected sample cells localizing in the indicated organ, and is normalized such that the % of control, untreated sample cells localized defines an RLR of unity. In recipients of MEL-14 pretreated cells, 52% of sample (TRITC-labelled) cells localizing in Peyer's patches bore residual MEL-14 antibody (82 of 159 cells counted), as determined by staining with FITC rabbit anti-rat Ig; and 77% (116/150) were MEL-14 positive when reincubated with MEL-14 followed by second stage antibodies.

conditions suggests that the putative receptor protein is not disulphide linked either to itself or to other polypeptides. The more rapid migration on SDS-PAGE in nonreducing conditions may be due to intra-chain disulphide bonding, which could produce a more compact protein structure. The absence of multiple coprecipitated polypeptides in this type of analysis does not, of course, rule out noncovalent homotypic or heterotypic association of these molecules on the cell surface.

Discussion

The antigenic determinant detected by the antibody MEL-14 is present on those normal lymphocytes and lymphoma cells which can bind peripheral lymph node HEV, and is absent from lymphoid cells which either do not recognize HEV or bind only to Peyer's patch HEV. No correlation is observed between MEL-14 determinants and the presence of other known cell-surface markers. MEL-14 specifically inhibits the lymphocyte-peripheral lymph node HEV interaction without affecting adherence to Peyer's patch HEV *in vitro*, and selectively interferes with the migration of lymphocytes into peripheral lymph nodes *in vivo*. Thus, it appears that the 80 and 92 K cell-surface structures detected by MEL-14 on normal lymphocytes and 38C13 cells either function as receptors for the specific binding of peripheral node HEV determinants, or are in close association (both physically and developmentally) with such receptors. More formal proof of a receptor function for the molecule(s) defined by MEL-14 would require demonstration that, as purified molecules, they bind specifically to peripheral lymph node (and not to Peyer's patch) HEV.

In vivo and *in vitro* studies on the selective migration and HEV-binding of small B and T lymphocytes^{18,19} and of immunoblasts^{20–26} to intestinal (mucosal) and peripheral (non-mucosal) lymphoid tissues have suggested at least two func-

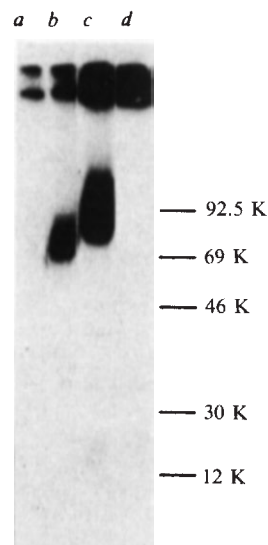


Fig. 4 SDS-PAGE of the molecules recognized by MEL-14 antibody on the surface of normal (C57BL/6 $\times$ BALB/c)F₁ mesenteric node lymphocytes and 38C-13 cells. After lactoperoxidase-catalysed surface radioiodination⁴⁸, 10^7 cells were solubilized with detergent and the lysates were clarified by ultracentrifugation as described by Witte *et al.*^{49,50}. Multiple 100- μ l aliquots of labelled material were incubated overnight at 4 °C in flexible microtitre plate (Cooke) wells which had been precoated with various affinity-purified rat monoclonal antibodies. (Affinity purification was accomplished on rabbit anti-rat Ig columns). 5–10 μ g/ml of each antibody in phosphate buffered saline was incubated in each microwell overnight at 4 °C. The wells were then washed with phosphate-buffered saline containing 1% bovine serum albumin. A more complete description of this technique will be reported elsewhere (manuscript in preparation). After four washes with 200 μ l of phosphate buffer containing 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.1 M NaCl, and 0.01 M sodium phosphate, pH 7.5⁵¹, bound material was eluted from the microwells with Laemmli sample buffer⁵² and analysed on a 9% SDS-polyacrylamide gel. ¹²⁵I-labelled molecular weight standards (NEN) were cytochrome *c* (12,000), carbonic anhydrase (30,000), ovalbumin (46,000), albumin (69,000) and phosphorylase B (92,500). The gel was dried and fluorographed on Kodak XAR-5 film for 4 days. Gel lanes: a, Normal (C57BL/6 $\times$ BALB/c)F₁ lymphocytes analysed with 11B-1, a control rat IgG2a monoclonal antibody which recognizes a specific determinant on BCL₁ (ref. 51) lymphoma cells (M. McGrath, personal communication) and b, MEL-14. c, 38C-13 cells analysed with MEL-14 and d, 11B-1. With shorter exposure times (1–2 days) the precipitates in lanes b and c were still observed as single diffuse bands.

tionally distinct migratory specificities mediated by selective recognition of HEV. The experiments reported here provide the first direct evidence for two antigenically distinct receptor specificities, one for peripheral lymph node HEV recognized by MEL-14, and one for Peyer's patch HEV which lacks MEL-14 determinants. Whether these determinants are located on separate molecules or on conformationally independent domains of a single structure is not yet known, but the existence of lymphomas which bind only a single HEV type would be consistent with the latter alternative only if selective inactivation of one or the other receptor domains could occur.

Several other groups have recently reported studies relating to the molecular mechanisms of lymphocyte-HEV interactions. Chin *et al.* have identified, in rat lymph, a factor of approximately 160 K apparent molecular weight which, when preincubated on rat lymph node sections, inhibits the subsequent adherence of rat lymphocytes to HEV^{27,28}. Precoating of lymphocytes with (Fab)₂ fragments from a conventional rabbit antiserum prepared against this factor resulted in inhibition of

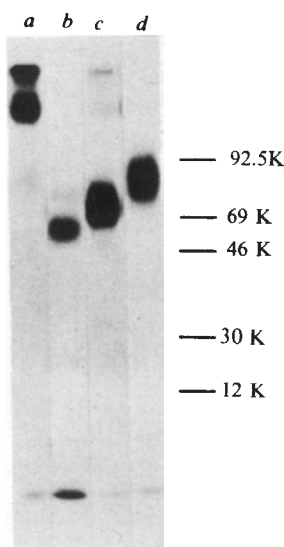


Fig. 5 The effect of reducing or nonreducing conditions on the electrophoretic mobility of the molecule recognized by MEL-14 on normal lymphocytes. Radioiodinated cell-surface molecules prepared as in Fig. 4 were isolated by overnight incubation at 4 °C with Affigel-10 beads (Biorad) which had been directly conjugated to either MEL-14 or 11B-5, a monoclonal antibody which binds to mouse IgM heavy chain (M. McGrath, personal communication). (Affigel-10 coupling to antibody was carried out in 100 mM HEPES buffer at pH 7.2 at 2 mg. of affinity purified antibody per ml of gel bed, with subsequent addition of glycine to block remaining reactive sites.) Radiolabelled material was eluted by boiling the beads 5 min in sample buffer⁵¹ with or without 2-mercaptoethanol and was analysed on a 9% polyacrylamide gel. The autoradiograph was exposed for 2 days. Gel lanes: a, 11B-5, nonreducing conditions; b, 11B-5, reducing conditions; c, MEL-14, nonreducing conditions; and d, MEL-14, reducing conditions.

lymphocyte binding to HEV and of lymphocyte migration *in vivo*²⁹. Identification of the lymphocyte surface determinants recognized by this antiserum and their structural relationship to the active moiety found in rat lymph have not yet been reported. Although the active factor reported by Chin *et al.* differs markedly in size and source from the putative HEV-

receptor recognized by MEL-14, their experiments independently support the notion that specific adhesion molecules mediate lymphocyte-HEV interaction.

The biochemical characteristics of the endothelial cell determinants which may be recognized by MEL-14 defined molecules are at present unknown, but recent experiments of Stoolman and Rosen³⁰ and Kieda and Monsigny³¹ suggest that they may contain an important carbohydrate component. Pre-treatment of rat lymphocytes with the polysaccharide fucoidin (but not with other polysaccharides) inhibits their adherence to lymph node HEV³⁰, as does addition of a synthetic neoglycoprotein, b-lactosyl-bovine serum albumin³¹. Indeed, the involvement of cell surface molecules with lectin-like activity has been described in the aggregation of teratocarcinoma cells³² as well as in other cell-cell adhesion models³³⁻³⁵. It remains possible, however, that these polysaccharide-containing substances inhibit lymphocyte-HEV binding by nonspecifically altering the lymphocyte membrane, rather than by interfering selectively with the specific lymphocyte surface elements involved.

Using another approach Andrews *et al.* have demonstrated that rat HEV cells actively take up ³⁵SO₄ and incorporate it into a glycosylated macromolecule³⁶ (possibly a glycolipid³⁷) that is released or secreted in noncovalent association with a pronase-sensitive component. Preliminary functional studies suggest that this complex may enhance lymphocyte-HEV binding *in vitro* (P. Andrews, personal communication), and induce lymphocytes to accumulate at intradermal sites of injection³⁸.

While certain types of homotypic cell-cell recognition may be due to like-like interactions at the molecular level, this is unlikely in the lymphocyte-HEV model as HEV cells do not appear to express significant amounts of the MEL-14 antigen (R. Reichert, personal communication). Although direct comparison of lymphocyte receptors for HEV with molecules mediating other examples of heterotypic cell-cell association may be unwarranted, it is possible that the MEL-14 defined structures may be part of a larger family of evolutionarily related molecules mediating many cell-cell recognition events necessary for the morphogenesis and function of tissues.

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Platelet-derived growth factor is structurally related to the putative transforming protein p28^{sis} of simian sarcoma virus

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A partial amino acid sequence of human platelet-derived growth factor, the major mitogen in serum for cells of mesenchymal origin, has been determined. A region of 104 contiguous amino acids shows virtual identity with the predicted sequence of p28^{sis}, the putative transforming protein of simian sarcoma virus (SSV). This similarity suggests a mechanism for transformation by SSV and other agents, involving expression of growth factors.

THE majority of cells maintained in tissue culture require serum to support efficient growth¹⁻⁷. The major polypeptide mitogen in serum for cells of mesenchymal origin is the platelet-derived growth factor (PDGF)⁸⁻¹⁰ (for a recent review see ref. 11). The precise physiological role of PDGF remains elusive. PDGF is stored in platelet α -granules^{12,13} and released locally during platelet activation when blood vessels are injured. PDGF is a potent chemoattractant for monocytes and neutrophils¹⁴ and for fibroblasts and smooth muscle cells¹⁵⁻¹⁷, and therefore may be involved in tissue repair processes. PDGF binds to specific saturable cell-surface receptors¹⁸⁻²¹ and stimulates tyrosine-specific kinase activity²²⁻²⁴ probably associated with a receptor molecule^{25,26}. Other early cellular responses include changes in ion fluxes²⁷, accumulation of cyclic AMP mediated by synthesis of E-type prostaglandins²⁸⁻³¹, changes in phosphorylation of intracellular proteins^{32,33}, stimulation of actin reorganization and membrane ruffling³⁴, modulation of epidermal growth factor binding^{35,36} and expression of specific genes^{37,38}. The significance of these changes for the mitogenic pathway remains to be elucidated.

Many transformed cells are able to grow in serum-free media or with reduced levels of serum, suggesting a decreased requirement for the serum growth factors^{3,39}. Transformed cells also may secrete mitogens such as multiplication-stimulating activity (insulin-like growth factors)^{40,41} and transforming growth factors^{42,43}, which do not appear to be structurally related to PDGF. However, a mitogen which seems to be structurally, functionally and immunologically related to PDGF has been partially purified from human osteosarcoma cells (U-2 OS)^{10,44}. In addition, the growth factor released by simian virus 40 (SV40)-transformed BHK cells^{45,46} also has PDGF-like properties⁴⁷. To further investigate the structural basis of the relationship between these tumour cell products, PDGF and other growth factors, we have determined a partial amino acid sequence for human PDGF.

Using these results, a computer search with the latest protein data base NEWAT of Doolittle⁴⁸ was initiated and revealed a region of virtual identity between a 90-residue stretch of PDGF and that of the predicted sequence of p28^{sis}, the putative transforming protein of simian sarcoma virus (SSV)⁴⁹. This striking homology suggests that the virus has acquired cellular sequences which encode a growth factor identical or very similar to PDGF and that expression of this protein mediates transformation by the virus.

Purification and characterization

The purification of PDGF from outdated platelet-rich plasma or from fresh platelets has been reported⁵⁰⁻⁵⁷. The methods

used take advantage of the extremely basic pI (9.8–10.2), hydrophobicity, heat stability and molecular size of PDGF. Unreduced PDGF has an apparent molecular weight (MW) on SDS-polyacrylamide gels of 27,500–31,000. Using gel permeation chromatography, PDGF has been preparatively separated into two chemically defined components (PDGF I and PDGF II, having MWs 31,000 and 28,000, respectively^{52,54,56}) which have essentially identical amino acid compositions but differ in covalently bound carbohydrate⁵⁴. Four components have been resolved on SDS-polyacrylamide gels (MWs 27,000, 28,500, 29,000 and 31,000)⁵⁵. Because PDGF is resistant to denaturation by SDS it has been possible to show co-migration of mitogenic activity with the 27–31,000-MW polypeptides eluted from gels run without reducing agents^{55,58,59}.

Following cleavage of the disulphide bonds of PDGF, mitogenic activity is lost and a number of polypeptides ranging in apparent MW from 17,500 to 11,000 are formed which have been partially resolved on SDS-polyacrylamide gels⁵³⁻⁵⁶ (see also Figs 1A, 2A) or by reverse-phase HPLC in pyridine formate buffers using propanol as organic modifier⁵⁷. The relative amounts and MWs of the polypeptides observed by these different techniques vary between preparations. Amino acid analyses have shown that PDGF has 18 cysteine residues which could be arranged as nine disulphide bonds⁵⁴. Thus, extensive cleavage by proteases could occur without changing the apparent MW of the unreduced molecule. As platelets are a rich source of proteases, these cleavages either may occur during purification or may be involved in activation of a PDGF precursor activity, as the proteolysed molecule is biologically active.

Component polypeptide fractionation

Our initial attempts to determine the amino acid sequence of PDGF showed that all preparations had at least five different amino-terminal sequences, and hence at least five different polypeptides were present in PDGF. HPLC techniques utilizing guanidine were used to overcome problems encountered in conventional fractionation techniques that are perhaps related to the hydrophobic and basic properties of PDGF.

Following full reduction and alkylation of disulphide bonds⁶⁰, the component polypeptide chains of PDGF were fractionated by either of two methods. On gel permeation HPLC in 6 M guanidine-HCl (ref. 61), PDGF II, purified from outdated platelets as described by Deuel *et al.*⁵⁴, yielded four size fractions having apparent MWs of 13,000, 8,840, 5,320 and 3,454 (Fig. 1B). The material in the 3,454-MW fraction was further resolved into six components (a–f) by reverse-phase HPLC using 0.1% trifluoroacetic acid with acetonitrile as the organic modifier⁶² (Fig. 1C). Alternatively, PDGF prepared from fresh

platelets according to a modification (see Fig. 2 legend) of the method of Johnsson *et al.*⁵⁷ was reduced and alkylated, and subjected to reverse-phase HPLC in 2 M guanidine-HCl, 1 M acetic acid. In accordance with previous findings⁵⁷, two groups of peptides were obtained, one eluting early (previously designated A) and the other eluting late (previously designated B) in the propanol gradient. Consecutive fractions from the HPLC chromatogram were analysed by SDS-gel electrophoresis (Fig. 2). Fractions 3 and 4 (Fig. 2B) were combined, subfractionated by gel permeation HPLC in guanidine-HCl and analysed by SDS-gel electrophoresis (Fig. 2C).

Amino acid sequence

Amino-terminal amino acid sequences were determined with a gas-phase sequencer assembled and operated as described by

Hewick *et al.*⁶³, using analytical techniques suitable for phenylthiohydantoin (PTH) amino acid quantification in the picomole range⁶⁴. Analysis of polypeptides obtained by HPLC from fully reduced and alkylated PDGF (as described in the legends to Figs 1 and 2) accounted for all the sequences found before fractionation. Figure 3 presents detailed sequence data for analysis of fractions from the PDGF preparation of Johnsson *et al.*⁵⁷; other sequence data are not presented here but have been reviewed. The major amino-terminal amino acid sequences obtained can be accounted for by the five peptides (I-V) shown in Fig. 4, together with a variant of peptide I which lacks five amino-terminal amino acids and a variant of peptide V which lacks an amino-terminal lysine residue. The apparent MWs of the fragments sequenced are indicated by the lengths of the dashed lines in Fig. 4. Sequence analysis of other fractions

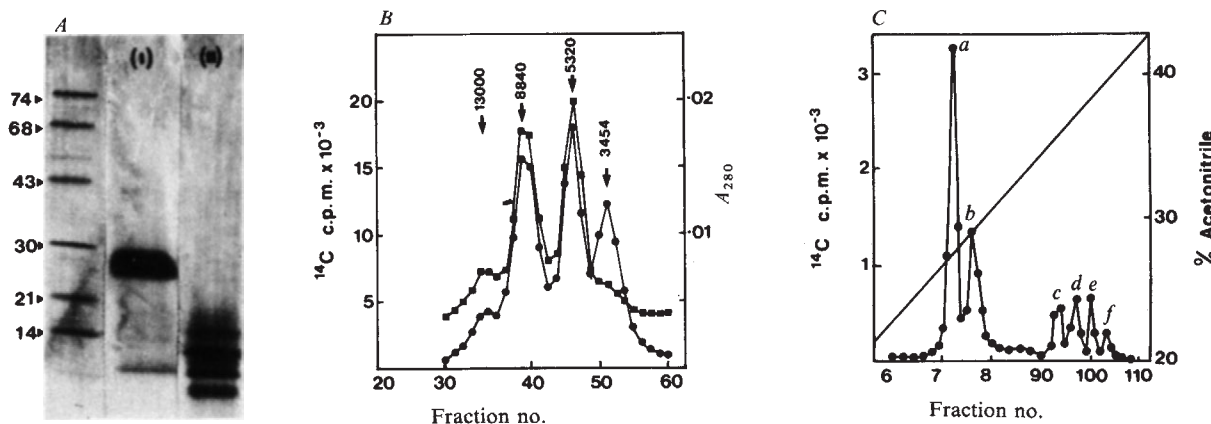
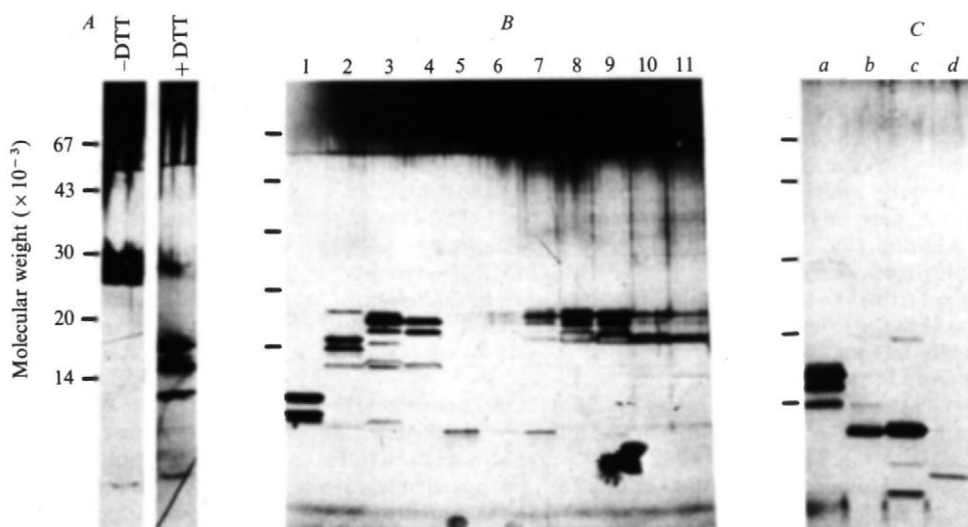


Fig. 1 Fractionation of component polypeptides of PDGF II by HPLC. PDGF II was purified by the method of Deuel *et al.*⁵⁴. **A** Shows an SDS-polyacrylamide gradient gel (5–22%) of PDGF II run in the absence (I) or presence (II) of dithiothreitol (DTT) and stained with Coomassie blue⁷⁶. Standards are shown in the left-hand lane; numbers refer to the molecular weight ($\times 10^{-3}$). PDGF II was fully reduced with DTT and cysteine residues were alkylated with ^{14}C -iodoacetamide (Amersham)⁶⁰. ● Indicates radioactivity; ■, absorbance. Peptides were fractionated by gel permeation in 6 M guanidine-HCl (Schwarz-Mann) at pH 4.5 with 0.1 M potassium phosphate buffer using a TSK SW 3000 column (0.7×60 cm; LKB) monitored at 280 nm and run at a flow rate of 0.5 ml min^{-1} . **B**, Molecular weights were determined using standard proteins including fully reduced and alkylated bovine serum albumin, ovalbumin, chymotrypsinogen and lysozyme by plotting $(\text{MW})^{0.555}$ against $K_{0.333}$ (ref. 61). The 3,454-MW fraction was then fractionated (**C**) by reverse-phase HPLC in 0.1% trifluoroacetic acid on a Synchropak RPP column (0.46×7.5 cm; Synchrom Linden, Indiana, USA) using a linear acetonitrile gradient from 1 to 60% (ref. 62). Column effluent was monitored at 206 nm. Material from the 8,840-MW fraction was found to contain peptides with identical amino-terminal sequences to those of peptides II and IV; peptide IV was recovered pure from the 5,320-MW fraction. Subfractions *a* and *b* of the 3,454-MW fraction were found to contain peptide V and fractions *c*, *d*, *e* and *f* were fragments containing the amino-terminus of peptide I.

Fig. 2 Analysis of component polypeptides of PDGF prepared by the method of Johnsson *et al.*⁵⁷, fractionated by HPLC. PDGF was purified and its constituent polypeptides separated as described by Johnsson *et al.*⁵⁷, with the modification that the solvent in the reverse-phase HPLC steps (using a Lichrosorb RP8 column, 0.4×25 cm, $5\text{-}\mu\text{m}$ particle size; Merck) was changed to 2 M guanidine-HCl, 1 M acetic acid. The columns were developed with gradients of propanol as before. The starting material analysed in the absence or presence of reducing agent is shown in **A**. Aliquots of the fractions from the chromatography were analysed on a 13–16% SDS-polyacrylamide gradient gel⁷⁶ which was developed with a silver stain⁷⁷ (**B**). Standard proteins are shown in the left-hand lane; numbers refer to the molecular weight ($\times 10^{-3}$). Fractions 3 and 4 were



pooled and further fractionated in 6 M guanidine-HCl, 0.1 M potassium phosphate, pH 4.5 on a TSK SW 3,000 column (0.7×60 cm; LKB) connected to a TSK SW 2,000 column (0.7×60 cm) at a flow rate of 0.1 ml min^{-1} . Four subfractions, *a*, *b*, *c* and *d*, were obtained; these fractions were characterized on SDS-polyacrylamide gels (**C**). Fraction *a* contained material having the amino-terminal sequence of peptide II, fraction *c* material with sequence of both peptides II and III for which the sequence data are shown in Fig. 3, and fraction *d* contained material with the sequence of peptide III. Fractions 10 and 11 were pooled and the sequence for peptide I was obtained. Fractions 8 and 9 were cleaved with cyanogen bromide and the resulting mixture sequenced. Results are described in the text.

revealed mixtures of polypeptides having one or more of the amino-terminal sequences indicated for peptides I–V in Fig. 4. In some cases the apparent MWs are lower than those shown by the dashed lines, indicating further carboxy-terminal cleavage of these peptides. Our results do not yet allow us to define the precise nature of these cleavages.

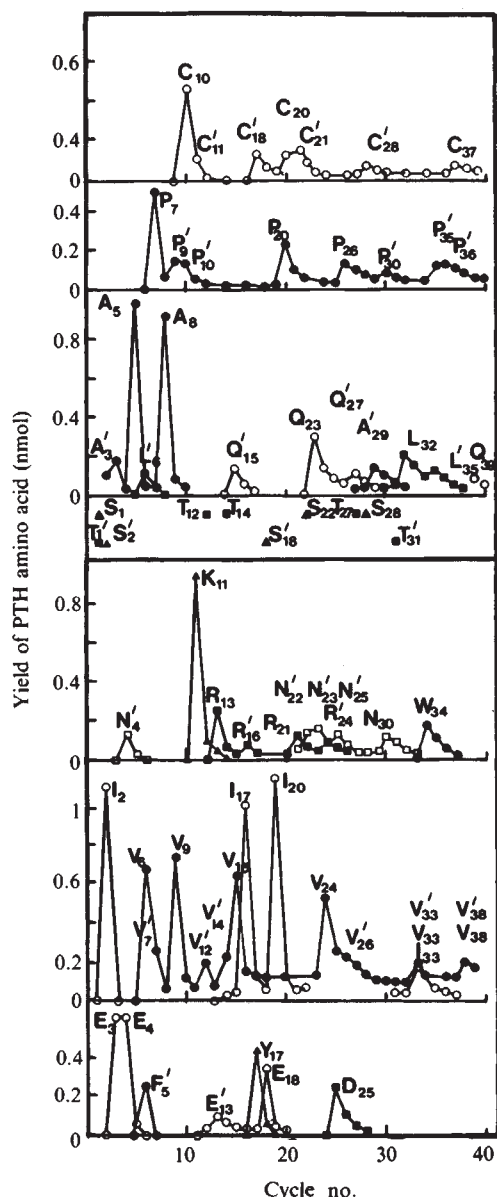


Fig. 3 Sequence analysis of PDGF fraction containing peptides II and III. Material from the Johansson *et al.*⁵⁷ preparation (fractionated and purified as shown in Fig. 2) was analysed. Two sequences were determined—a major and a minor sequence. The latter is indicated by the prime marks following the relevant amino acids. Sequence determination was carried out using a gas phase sequencer assembled and operated as described by Hewick *et al.*⁶³. PTH amino acids were analysed by HPLC using a Zorbax C8 column (4.6 mm × 25 cm; Dupont) at 43 °C with a linear gradient over 8 min of acetonitrile from 24% to 38% at a flow rate of 2 ml min⁻¹ using a 0.009 M sodium acetate buffer pH 4.1 as described by Waterfield *et al.*⁶⁴. A Waters system including two M6000A pumps, a WISP autoinjector and system controller with a Beckman Model 160 detector was used. The recovery of PTH amino acids at each degradative cycle was measured using an integrating recorder (Waters Data module). The analyses obtained for serine and threonine were not accurately quantifiable due to the presence of multiple peaks obtained during analysis of the PTH amino acids. The presence of these amino acids is thus indicated without yield data. The assignment of these residues to the major and minor sequences was made using semi-quantitative recovery data based on peak heights rather than areas.

Structure of PDGF

The amino-terminal sequences of peptides I and II are homologous and appear to differ in length by six amino acids. The aligned sequences share 12 identical residues and show 5 conservative substitutions over the 31 residues of peptide I and 25 residues of peptide II. Cyanogen bromide cleavage of peptide I resulted in cleavage both at methionine 12 and at a tryptophan residue, revealing a sequence identical to that of peptides II, III and IV extending from the tryptophan residue at position 34 of peptide II towards the C-terminus. As no contiguous sequence has been obtained between residue 31 of peptide I and this particular tryptophan residue, it is not clear whether peptide I is homologous (or indeed has the same number of residues) over the region corresponding to amino acids 26–34 of peptide II. Alignment of the sequences of peptides I–V against the amino acid sequence of p28^{sis}, the predicted translation product of the *v-sis* gene of simian sarcoma virus⁴⁹ (see below and Fig. 4), reveals the possible origin of the proteolytic cleavages and peptide fragments found in PDGF. Thus, cleavage at residues homologous to the basic amino acids 66, 99, 146 and 172 of the predicted sequence of p28^{sis} would generate peptides I, III, IV and V of approximately the experimentally observed molecular weights. Peptides III and IV have been found to differ only at residue 2 and could be derived from peptides I and II. Peptide V is clearly derived from a region C-terminal to peptides III and IV because it shares considerable identity with the *v-sis* predicted amino acid sequence in this region. If PDGF also includes the carboxy-terminal basic region of the *v-sis* product, which it may well do as this could account for the *pI* of 9.8–10.2 reported for PDGF¹¹, then many additional tryptic type cleavages could generate a multitude of different polypeptide chains. We have been unable to find sequences corresponding to either residues 1–66 or as yet 172–226 of the *v-sis* product. It is possible that the failure to detect cleavage products in the highly basic C-terminus could be due to technical problems. It seems less likely that PDGF extends over the N-terminal residues 1–66 of p28^{sis} (containing three methionine residues), because a search for methionine-containing peptides derived from PDGF has not revealed any other peptides containing a methionine other than that in peptide I.

The largest component polypeptide chains of PDGF found in this work having the amino-terminal sequences of peptides

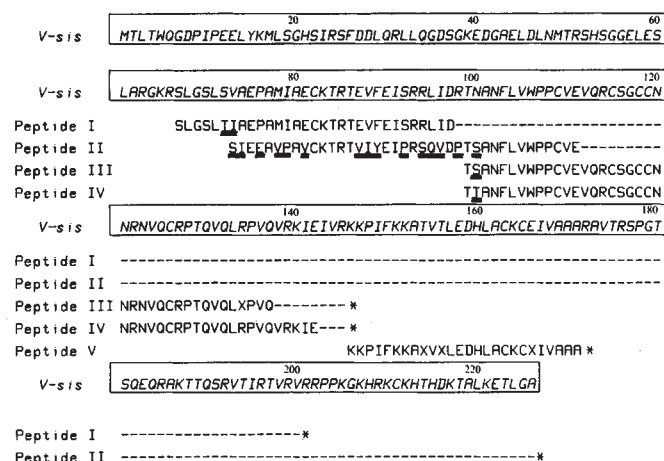


Fig. 4 The relationship between the amino acid sequence of PDGF chains and the predicted amino acid sequence of *v-sis*. The predicted amino acid sequence of *v-sis* starting from the presumptive initiation codon at nucleotide 3,657 and continuing to the end of the open *v-sis* reading frame of the SSV sequence of Devare *et al.*⁴⁹, is shown. The partial amino acid sequences of peptides purified from PDGF are shown. The approximate length of these peptides is indicated by dashed lines terminating in * at their possible carboxy-terminus. Boxed residues within the peptide sequences indicate differences between the peptide sequence and that of the *v-sis* predicted amino acid sequence.

I and II have apparent MWs of 14,500 and 17,500. It has previously been suggested that PDGF contains two polypeptides (A and B) linked by disulphides⁵⁷, one of which is more susceptible to proteolytic degradation. The data presented here could support such a model with two distinct but homologous chains, A and B, chain A corresponding to peptide II and chain B to peptide I. It is also possible that the 31 amino-terminal residues of peptide I determined are located at the amino-terminus of peptide II, and the origin of all the chains found in PDGF could arise from cleavage of a single precursor polypeptide of 21,000 MW. However, this seems less likely considering molecular weight estimates for native PDGF¹¹.

During the preparation of this article, Antoniades and Hunkapiller⁶⁵ reported less extensive sequences of human PDGF than those reported here and with three important differences. In their sequence of chains 2a and 2b (which are the same as peptide I and a variant of peptide I), Antoniades and Hunkapiller found glutamic acid at position 20 and cysteine residues at positions 24 and 26. In our work we found threonine, glutamic acid and serine at these positions—these residues are identical with those predicted from *v-sis* at homologous positions. The origins of these differences are unclear.

Relationship between PDGF and p28^{sis}

The PDGF amino acid sequence and the sequences in the 1983 NEWAT protein data base of Doolittle⁴⁸ were compared using the rapid searching techniques of Wilbur and Lipmann⁶⁶. A stretch of 104 residues was found to be shared by PDGF and the amino acid sequence of p28^{sis} as predicted from the DNA sequence of the *v-sis* region of the SSV genome⁴⁹. No obvious relationships of the PDGF sequence to those of other growth factors or oncogenes was established in this search; however, this data base is necessarily some months behind published data and further searches are in progress. Recently, Besmer *et al.*⁶⁷ have shown that the Parodi-Irgens feline sarcoma virus has an oncogene related to that of SSV which is expressed as a fusion protein. No sequence data are yet available for this protein or the viral genome.

The precise amino-terminus of the *v-sis* gene product is unknown. Figure 4 shows the predicted amino acid sequence covering a region from the most likely initiation point (the ATG at position 3,657 in the sequence of the SSV genome⁴⁹) to the end of the open reading frame of *v-sis*, a stretch of 226 residues. Figure 4 also shows the amino acid sequences of peptides I to V aligned with that of the predicted *v-sis* product.

A region of virtual identity exists between the partial amino acid sequences determined for peptides I, III, IV and V and that of the protein p28^{sis} predicted from the *v-sis* sequence over a region extending from p28^{sis} residue 67 to 171. Apart from the small gaps (7 residues) which (due to technical problems) we have been unable to establish, only three substitutions have been found, all of which are conservative and could represent the difference between human PDGF and the SSV acquired monkey cellular sequences. The amino-terminal sequence of peptide II cannot be encoded by *v-sis*, but is homologous to the same region of the *v-sis* product as the amino-terminus of peptide I (13 identities and 5 conservative substitutions).

The region of virtual identity between the predicted sequence of p28^{sis} and a region of one peptide found in PDGF strongly suggests that SSV has acquired cellular sequences which encode part of PDGF or of a member of a family of very similar gene products. Cellular sequences encoding the amino-terminus of peptide II have not been acquired by the virus. These sequences may be encoded within the same cellular gene but not acquired by the virus or may be derived from a separate gene. It is also possible that in SSV these cellular sequences have become rearranged to delete that part which would code for the amino-terminus of peptide II, substituting the amino-terminus of peptide I.

v-sis-related sequences have been located in the human genome at a single locus (*c-sis*) which is interrupted by at least

four regions of nonhomologous cellular sequences^{68–70} on chromosome 22 (22q11 > qter)⁷¹. The sequence of the first exon in *c-sis* has been determined⁷⁰ but the sequence of the other regions is required to clarify further any relationship between *c-sis* and PDGF. It has been shown that five of six sarcoma cell lines and three of five glioblastoma lines contain a single 4.2-kilobase (kb) transcript (together with an occasional second RNA species present at a lower level) not found in SSV non-producer cell RNA, normal human embryonic fibroblasts, carcinomas or melanomas⁷². A similar size transcript has also been found in the human T-cell lymphoma cell line (HUT 102) which is known to produce the retrovirus human T-cell leukaemia (lymphoma) virus⁷³.

The 4.2-kb RNA could clearly encode a much larger polypeptide than PDGF, suggesting the existence of a large precursor similar to that recently reported for epidermal growth factor which is 53 amino acids long but may be cleaved from a precursor of 1,168 amino acids⁷⁴.

The putative transforming protein of SSV, p28^{sis}, has been shown to be a polypeptide with an apparent MW of 28,000 (ref. 75), although it is not clear whether gels were run with reducing agents. Glycosylation of residue 48 of p28^{sis}, together with other post-translational processing, may occur. The predicted MW of a peptide extending from residue 67 (that is, the starting point of peptide I) to residue 226 is 17,500, similar to the size of the largest fragment of reduced PDGF.

v-sis has a long open reading frame which encodes p28^{sis} and this sequence could clearly encode peptide I of PDGF but not the amino-terminus of peptide II⁴⁹. If PDGF has two distinct chains, the virus may have acquired sequences for only one of these. It is possible that this chain then accounts for the growth-promoting activity. It is also conceivable that the putative second polypeptide chain II of PDGF is encoded and expressed by the host cell.

PDGF oncogenes and transformation

Abnormal expression of PDGF or of a related protein through transformation by SSV could lead to uncontrolled growth by transformed or tumour cells through secretion of the growth factor or by a direct intracellular effect. As p28^{sis} is an *env-sis* fusion protein, it is possible that the *env* sequences which normally allow membrane insertion of *env* (in the absence of a C-terminal transmembrane region) lead to secretion of the transforming protein. Thus, cells having PDGF receptors and capable of mounting a mitogenic response to PDGF, such as mesenchymally derived cells, could respond and become constitutively activated through the receptor-mediated response. Alternatively, normally PDGF-unresponsive cells infected by SSV could respond if the mitogenic signal was intracellular and bypassed the need for receptor interactions at the cell surface. Equivalent results could follow the increased production of the *c-sis* product by a variety of mechanisms including changes in the control of *c-sis* transcription and mRNA translation. It is clear that certain specific tumour cells⁷² of mesenchymal origin do have detectable levels of an RNA species which hybridizes to *v-sis* and which could be the product of *c-sis*, the putative gene encoding PDGF. These are cells which might normally be expected to respond to PDGF. Further studies will demonstrate the relationship between the PDGF-like factors produced by human osteosarcoma cells⁴⁴ and SV40-transformed BHK cells⁴⁷, p28^{sis}, and the product of *c-sis*. It remains to be seen if p28^{sis} is a growth factor, whether it is secreted and if it binds to the PDGF receptor to induce a mitogenic response.

The degree of involvement of these molecules in transformation should provide clues to the disturbance of growth control in cancer cells. The search for the function of other oncogenes may well unravel further interactions with the mitogenic cascades induced by growth factors.

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LETTERS TO NATURE

Inflation does not explain time asymmetry

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Davies¹ has argued that the inflationary cosmological scenario²⁻⁵ provides a natural explanation for the time asymmetry of the Universe. Here I dispute this argument by noting that the inflationary scenario implicitly invokes time asymmetry with the assumption of an absence of initial spatial correlations. No scenario based on charge-parity-time (CPT)-invariant dynamical laws can explain the time asymmetry apart from postulating or explaining these special initial conditions, as Penrose^{6,7} has emphasized.

The time asymmetry of the Universe is expressed by the second law of thermodynamics, that entropy increases with time as order is transformed into disorder. The mystery is not that an ordered state should become disordered but that the early

Universe apparently was in a highly ordered state⁷. In the standard hot big-bang model, the matter (nongravitational) fields in the early Universe were not highly ordered, but the gravitational field was⁸. Penrose^{6,7} pointed out that the gravitational entropy would have been enormously higher had the early Universe been composed of enormous black holes. Thus the mystery is why the early gravitational field was so smooth.

Davies¹ argued that this mystery is explained in the inflationary scenario²⁻⁵ in which the gravitational repulsion of a false-vacuum energy density caused the early Universe to undergo exponential expansion. During this expansion, any anisotropies and inhomogeneities would asymptotically diminish exponentially (refs 9, 10, and A. A. Starobinsky, personal communication) so the gravitational field would approach the smooth de Sitter state. The argument is that this would make the gravitational field highly ordered by the time of the phase transition to a high-entropy state for the matter, so that the resulting hot Universe would then evolve as postulated in the standard big-bang model.

However, it is begging the question of time asymmetry to assume that this asymptotic decaying behaviour is what dominates the evolution of the gravitational irregularities during the false-vacuum phase. If the irregularities are viewed as perturbations of a background de Sitter space, the complete temporal

and spatial homogeneity of the latter implies that there is no *a priori* reason for supposing the perturbations get smaller with time. Nothing in the CPT-invariant dynamical equations implies this time asymmetry.

Of course, it is natural to assume that the initial perturbations spread out with time and get weaker rather than come together and get stronger, but this is a thermodynamic assumption. This unexplained time-asymmetric assumption is only 'natural' because that is how we see nature behave. We do not naturally see highly focused incoming radiation, so we postulate a time-asymmetric second law of thermodynamics to exclude it. The inflationary scenario relies on this assumption and is consistent with it, but inflation does not explain it.

The time asymmetry of the second law of thermodynamics may be elegantly expressed by the law of conditional independence¹¹ and its consequence that systems isolated in the past are uncorrelated. For a globally hyperbolic spacetime with an initial singularity (such as the big bang), this law is equivalent to the assumption that the Universe began with all spatially separated regions uncorrelated. Such an assumption makes the focusing of perturbations statistically improbable, so in an exponentially expanding phase the irregularities would tend to spread out and get weaker as the inflationary scenario assumes.

The unexplained mystery is why all spatially separated regions were apparently uncorrelated at the beginning. It would seem that they could be uncorrelated at any particular time, although interactions would generally induce correlations at all other times. Even more likely *a priori* would be for separate regions always to be correlated.

To illustrate how restrictive the second law is when expressed as an absence of initial spatial correlations, count parameters for allowed and disallowed quantum states in a schematic finite-dimensional model for the big bang. Partition an initial hypersurface (at, say, the Planck time or expansion rate) into n disjoint spatial regions, and consider the quantized matter and gravitational fields in each region as making up a quantum system with Hilbert-space dimension D . (D would ostensibly be infinite for this quantum-field-theory system, but one could imagine restricting the states to a finite D -dimensional subspace for some nonthermodynamic reason, for example to restrict the local energy.) The joint system of the n regions then has Hilbert-space dimension D^n , and its density matrix is an hermitian positive-semidefinite unit-trace $D^n \times D^n$ matrix, which thus has $D^{2n} - 1$ real parameters. Now these regions are uncorrelated in the quantum sense if their joint density matrix is the tensor product of the density matrices of the individual regions. The individual regions have density matrices with $D^2 - 1$ parameters each, so an uncorrelated joint density matrix has $n(D^2 - 1)$ parameters, which for $D > 1$ and large n is enormously less than $D^{2n} - 1$. Thus the thermodynamic restriction to an uncorrelated initial state selects a subspace of far fewer parameters than those of the most general allowed states without this restriction. The unexplained mystery of the second law is the rationale for this restriction.

It is true that the inflationary scenario may allow the Universe to begin with a large amount of arbitrariness, as Davies¹ asserts, in that it may not depend crucially on the individual density matrices of the disjoint regions. This freedom corresponds to the $n(D^2 - 1)$ parameters of the uncorrelated joint density matrix. But this freedom is minuscule compared with the freedom of a completely arbitrary joint density matrix.

An objection to this parameter-counting argument for the specialness of the second law is that our observations certainly do not prove that the Universe began spatially uncorrelated. Our finite observations have merely ruled out the finite number of conceivable correlations that would have produced observed antithermodynamic effects, such as focused incoming (advanced) radiation. A large class of initial correlations would doubtless have produced no such noticeable effects. Indeed, we do not even have direct evidence that the second law applies at all far away from either our past light cone or the world line of our Galaxy, so one could argue that it is rash to postulate a

universal second law for the entire Universe. If one refuses to assume a very special initial state for the Universe, one is led to the hypothesis that the order we see is a gigantic fluctuation. But then there is no reason to predict that future observations will continue to reveal more order. The fact that we remember being successful in past predictions that the order will persist has led most people to postulate that the second law in some form is universal, even though our observations can never prove that. Without knowing precisely what the second law means, it is an even bolder step to formulate it as the absence of initial spatial correlations, but that at least is one fairly definite formulation that may be defended on the grounds of simplicity. (Of course, this formulation cannot be made very precise without a theory of quantum gravity to describe the state on the initial hypersurface or whatever it is that replaces this spacetime description.)

But even if the second law should be formulated in a weaker form than the absence of initial spatial correlations, it is clearly a strong selection principle on the state of the Universe. There is no mechanism known as yet that would allow the Universe to begin in an arbitrary state¹ and then evolve to its present highly-ordered state.

If the inflationary scenario must invoke without explanation some thermodynamic assumption such as an initial absence of spatial correlations, one may ask whether certain other mysteries explained by the inflation would be consequences of this assumption even without inflation. It appears that the homogeneity and isotropy of the Universe may be examples of such consequences.¹² Because the gravitational field is coupled to matter through its stress-energy tensor, inhomogeneities in the matter must be accompanied by inhomogeneities and anisotropies in the gravitational field and generally by a non-vanishing Weyl tensor. If these nontrivial gravitational fields were spatially extended at the beginning, this would seem to require spatial correlations that the thermodynamic assumption would exclude. (Note that in this view a Friedmann cosmology has a trivial gravitational field that does not show any spatial correlations.) In other words, Penrose's suggestion^{6,7} of an initially vanishing Weyl tensor might be a consequence of the second law of thermodynamics as formulated above. Then the observed high degree of homogeneity and isotropy of the Universe would be 'explained' by the unexplained second law even without inflation.

(This is not to say that the inflationary scenario explains nothing, for it is apparently the best solution of the cosmological monopole problem¹²⁻¹⁴. Note that this problem arose in the standard big-bang model precisely because it was assumed that the Higgs field was spatially uncorrelated initially.)

It should be clear that any special characteristic of the state of the Universe, such as its time asymmetry, cannot be explained by CPT-invariant dynamical laws alone. Without some restriction on the state, one could imagine any arbitrary conditions today (large inhomogeneities, anisotropies, and so on) and then simply evolve these backward in time by the dynamical laws to get the corresponding initial conditions needed to produce them. It seems likely that most of these past histories would not include an inflationary phase, just as in the opposite direction of time a recontracting Universe is unlikely to undergo deflation¹. Thus one could say that the existence of an inflationary phase in the past puts strong limits on the present conditions of the Universe, but a demonstration of this fact would not be an explanation for these conditions, since it would leave unexplained why inflation occurred at all, much less why the inflationary phase followed the second law of thermodynamics.

Recently there have been some attempts to derive a preferred state for the Universe by a suitably restricted Euclidean path integral^{15,16} or by quantum tunnelling from 'nothing'^{17,18}. At present only some crude schematic characteristics of such a state can be deduced from necessarily highly approximate calculations, so it is not yet known whether these methods can explain the observed characteristics of the Universe and in particular its thermodynamic behaviour. However, it does seem clear that

only by some bold new method such as this that goes beyond mere dynamical laws can we have any hope of explaining the time asymmetry of the Universe.

It is possible that if some such method can give a preferred state for the Universe, the inflationary scenario may then have a role rather like what Davies envisages. However, there seem to be three essential elements that remain to be demonstrated for this to work: (1) The quantum analysis should somehow make it more likely for the Universe to start out small (such as, in Planck volumes at the Planck time or thereabouts), despite the enormous number of larger configurations that appear to be available. (2) It should be shown that a small Universe necessarily has low entropy. (A conjecture would be that on any spatial hypersurface in which the orthonormal spacetime curvature components are smaller than the Planck values, the entropy is less than some simple function of the number of field species present times the volume in Planck units: this inequality may be approximately saturated for the Friedmann–Robertson–Walker cosmologies at the Planck density.) (3) The inflationary phase should expand the volume of the Universe to a large

value and then end in a phase transition which somehow manages to increase the matter entropy enormously without increasing the gravitational entropy by a comparable amount. So far none of these three elements have been clearly demonstrated, but if analyses can be developed to show them, then we might have an explanation for the second law of thermodynamics.

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Constraints on γ -ray bursters from soft X-ray transients

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Perhaps the most enigmatic and elusive of the short time scale astrophysical phenomena observed so far are the γ -ray bursts discovered in the Vela satellite data in 1973¹. After a decade of observational and theoretical work, there is still no agreement on a model for the radiation detected or for the type of astronomical system responsible. We have conducted a search of the Columbia Astrophysics Laboratory's Einstein imaging proportional counter (IPC) database for possible X-ray counterparts to these transient events. The limiting sensitivity was $\sim 10^{-10}$ erg cm⁻² with a total exposure of $\sim 3 \times 10^6$ s divided amongst $\sim 10^3$ $1^\circ \times 1^\circ$ fields. Four events with a pointlike spatial distribution were detected but their extremely soft spectra ($kT \leq 0.1$ keV) makes them unlikely candidates for γ -ray burst counterparts. We use our results here to place limits on the X-ray and γ -ray luminosity ratio and log (number) N –log (flux) S relation for γ -ray bursts, and comment on the constraints placed on models for their origin.

γ -Ray bursts have been classified into three main categories²: those for which the 5 March 1979 transient is the prototype³, those like the 20-min transient observed by Jacobson⁴, and 'classical' γ -ray bursts. The latter, encompassing most of the several hundred events observed to date, are characterized by a very short risetime (< 1 s), a FWHM from 20 ms to 2 s and a total duration of 5–70 s (refs 2, 5).

The decade-long search for a satisfactory model of the burst phenomenon has been severely hampered by the lack of positive identification of the burst sources in other spectral bands despite extensive searches^{6–9}. Only recently has evidence for counterparts in the optical¹⁰ and X-ray regimes^{11,12} been proposed. Mitrofanov¹³, using the observed γ -ray burst rate from the Konus satellite, has predicted that several dozen X-ray counter-

parts should be detectable in the Einstein Observatory database. In addition, several theoretical models for the bursts predict associated events in the X-ray regime with energies comparable with those in the γ -ray band^{14,15}.

Our search for short time scale transients covered the data accumulated as part of the Columbia Astrophysics Laboratory's Einstein programme of IPC observations. The instrument¹⁶ has an energy range of 0.1–4.0 keV, an effective area of ~ 100 cm², and a $1^\circ \times 1^\circ$ field of view with a spatial resolution of 1 arc min. Approximately 3×10^6 s of IPC data were available; the distribution of sky coverage as a function of galactic latitude is summarized in Table 1. The data were conveniently formatted in $\sim 8 \times 8$ arc min blocks with 10 μ s time resolution and a mean background counting rate of 0.05 counts s⁻¹ per block. Thus, a 10-s integration most frequently yields values of 0 or 1 counts per block. Assuming Poisson statistics we can calculate a threshold number of counts, n , dependent only on the mean counting rate in each individual field, such that the probability of seeing $\geq n$ counts is, say, once every 10^6 trials. For example, for a mean of 0.5 count per trial, corresponding to a 10-s integration, $n = 6$. Thus, for a typical observation of length $\sim 2,000$ s, we expect one false alarm for every ~ 100 $1^\circ \times 1^\circ$ fields. A comparison of a Poisson distribution with the observed distribution of counts per block for a 20,000-s exposure is shown in Fig. 1; the close agreement of the two distributions in the low-probability tail confirms the applicability of our threshold setting technique.

The 10-s integration period is optimum for several operational as well as astrophysical considerations. The reality of an event containing fewer than 7 or 8 photons is, for a detector with finite background, statistics-limited, so a shorter integration time is of no advantage. On the other hand, an event of the same flux lasting more than ~ 50 s (40 total counts) would emerge above the background as an identifiable source in the integrated image and, thus, would have been catalogued by the automatic source detection algorithm and tested for variability. We checked ~ 500 such new sources discovered in our database and, while a few examples of clear variability emerged, none of the sources either appeared or disappeared on time scales shorter than the observation ($> 10^3$ s). Finally, ~ 10 s is the

Table 1 Sky coverage for the soft X-ray transient survey

Galactic coordinates		Time	Mean stellar	Predicted no.	
$ b $	l	(s)	density ($M_{\odot} \text{ deg}^{-2}$)	$E_{38} = 1$	0.1
0-1	$\pm 1^{\circ}$	5,880	2.5×10^9	0.22	7.1
0-3.5	$\pm 1 - \pm 3.5$	11,730	2.1×10^9	0.37	11.8
0-7	$\pm 3.5 - \pm 7.0$	31,240	2.3×10^8	0.11	3.5
0-15	$\pm 7.0 - \pm 15.0$	10,570	1.5×10^7	0	0.08
0-7	15-345	386,640	2.3×10^6	0	0.43
7-15	15-345	263,120	5.7×10^4	0	0
15-30	0-360	426,930	2.8×10^4	0	0
30-60	0-360	1,125,510	2.5×10^4	0	0.01
60-90	0-360	331,060	8.6×10^3	0	0
Galaxy total		2.7×10^6		0.70	23
LMC total		2.6×10^5	2.5×10^8	0.98	31

shortest time scale likely to be appropriate for γ burst counterparts or analogues.

Field thresholds were violated several hundred times in our search of the entire database. Most of these triggers resulted from the presence of a real X-ray source in the field which raised the local background counting rate; these candidate bursts were easily eliminated. The remaining events fell into two classes: those in which excess counts were observed simultaneously at several widely separated locations in the field and those in which a single 10-s spatial block exceeded the Poisson threshold. The first class, in which the whole counter 'lights up', clearly does not represent a celestial X-ray event; these events may be attributed to counter malfunction or the deposition of energy from a cosmic ray (or, conceivably, a burst of γ rays) which entered through the wall of the counter. We were

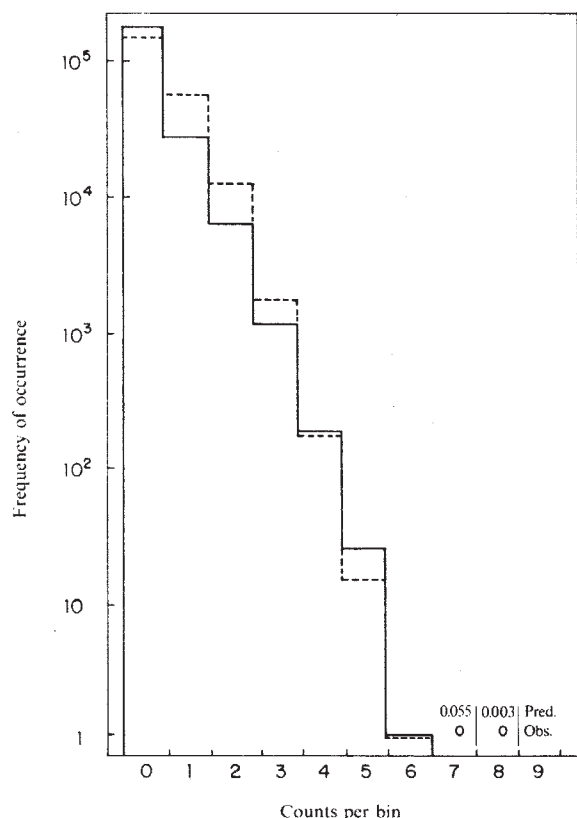


Fig. 1 The distribution of the observed frequency of occurrence of X-ray counts per 8.5×8.5 arc min bin per 10 s interval for a 17,650-s IPC exposure (solid histogram). This is compared with the expected Poisson distribution for a mean count rate per bin of 0.41 per 10 s interval (dashed histogram). The agreement at low count rates is marginal due to various nonstatistical effects (such as counter edge effects, sources just below threshold), but the agreement in the low-probability tail gives us confidence in the threshold setting techniques we have adopted in searching the data for soft X-ray bursts.

unable to find any coincidences between these events and previously published γ -ray burst times^{3,5,6,17}

The second class of events involved a single location with counts higher than the threshold for <20 s; 24 such events were found and were examined for spatial extent, time history and spectral information. All but four cases were eliminated because the spatial distribution of the detected photons failed to conform to the point source response function of the IPC; these 20 events are attributable to the 10^{-6} – 10^{-7} tail of the probability distribution for random occurrences or to instrumental idiosyncracies. The four remaining cases contained 29, 20, 13 and 9 counts, respectively, all falling within the expected radius for an imaged point source. All show a very soft spectrum with all counts falling in pulse height channels corresponding to photon energies of <0.75 keV. They range in duration from 1.25 to 10 s; a plot of the strongest burst is shown in Fig. 2.

We have checked several possibilities in an attempt to assign these events to instrumental or environmental phenomena but have been unable to do so. Redundant telemetry streams eliminate the possibility of a data transmission or storage error; in fact, examination of the primary data stream for the strongest burst revealed that the counting rate we observed was limited by instrument hardware and software filters—the true flux was ≥ 100 counts integrated over the burst. No previously recognized instrumental artefact fits the characterization of these events. Three of the four events did occur between 25 and 35 arc min from the telescope axis (although in different quadrants of the detector); this is not particularly surprising, however, in view of the facts that: (1) the central two to four blocks (up to 16×16 arc min) were often eliminated from the search because of the presence of the target source; and (2) the area in an annulus from 25 to 35 arc min is roughly equal to that over the rest of the counter. Thus, while we cannot categorically exclude a noncelestial origin, we provisionally accept the four events as real. A reasonably definitive test of this view is readily available: the entire Einstein IPC database contains ≥ 10 times the sky coverage of our data set and could be easily searched during reprocessing (with $<1\%$ overhead) for additional examples of the phenomenon.

The softness of the spectra of these events makes it unlikely that they are associated with γ -ray bursts (for example, no simultaneous event was recorded in the monitor proportional counter (2–20 keV energy band) at the time of the large burst). Our result of primary importance is the limit placed on proper-

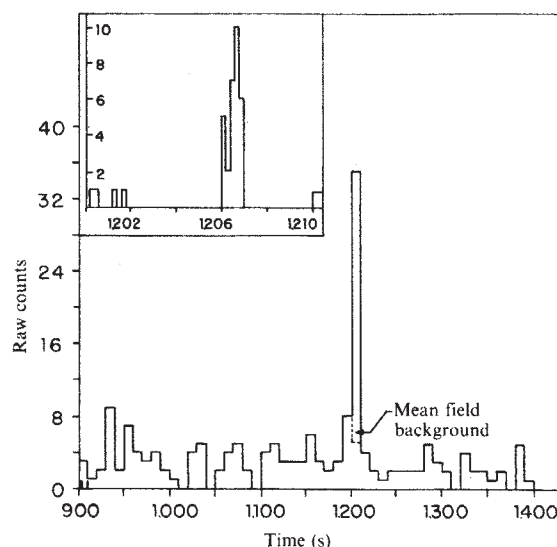


Fig. 2 The light curve in the 0.1–4.0 keV band of the strongest detected burst. Each 10-s bin (labelled from the start of the observation) represents the number of photons detected within a radius of 5 arc min centred at the burst location. The background rate during the burst interval as determined from an average over the entire $1^{\circ} \times 1^{\circ}$ field of view is shown. The inset shows the time structure of the burst at a resolution of 0.2 s.

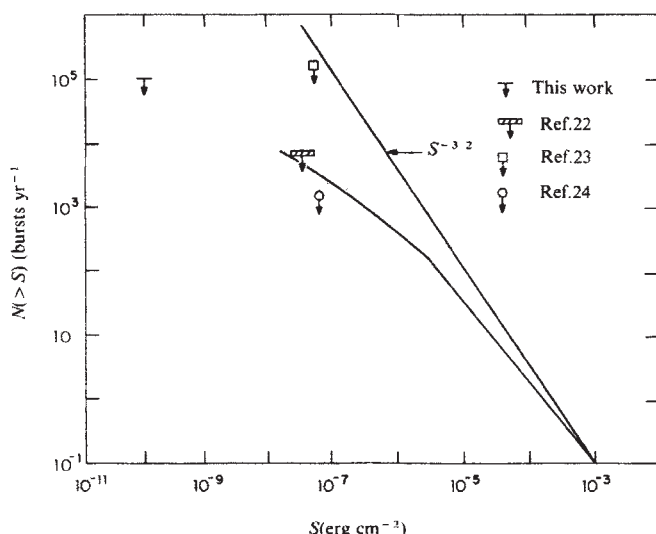


Fig. 3 The log N -log S distribution of γ -ray bursts²⁰. The straight line corresponds to an isotropic distribution ($S^{-3/2}$), while the lower curve corresponds to a disk distribution with a scale height of 400 pc (ref. 21). Representative upper limits from previous γ -ray work in the 10^{-7} erg cm $^{-2}$ flux range are noted. An upper limit derived from this work, assuming $I_x \sim L_x$ is also shown.

ties of γ -ray bursts. Mitrofanov's¹³ analysis of the Konus data, coupled with his assumption that the density of burst sources is proportional to the density of stars in the Galaxy, yields the following relation for the expected number of events, N , in such a search:

$$N = 4.8 \times 10^{-7} E_{38}^{-3/2} M_{\odot}^{-1} \text{ yr}^{-1}$$

where E_{38} is the mean burst energy in units of 10^{38} ergs. We have calculated the total stellar mass along each line of sight in our survey by computing the volume of the Galaxy searched in each direction and multiplying by the densities expected in these directions. The densities are taken from Oort's¹⁸ model for the central 1 kpc of the Galaxy and from Schmidt's¹⁹ model for the outer parts of the Galaxy. (To normalize the two models to the same total galaxian mass of $2 \times 10^{11} M_{\odot}$, the values in Schmidt's model were multiplied by a factor of ~ 3 .) In the case of the pointings towards the Large Magellanic Cloud (LMC), a mean surface density of $2.8 \times 10^8 M_{\odot} \text{ deg}^{-2}$ was adopted²⁰.

We have convolved these mass estimates with our sky coverage as given in Table 1. If the X-ray and γ -ray luminosities of bursts are comparable as has been suggested by recent models, we predict that, for $E_{38} = 1$, we should have seen one burst from the Galaxy and another from the LMC; for $E_{38} = 0.1$, the predicted rates are 23 and 31, respectively. We observed none.

This result may be interpreted in two ways. If $E_x/E_{\gamma} \sim 1$, the lack of observed bursts provides a lower limit to the mean γ -ray luminosity: $E_{\gamma} \geq 10^{38}$ erg s $^{-1}$. It also helps to constrain the log N -log S distribution for low fluxes. Our limit of $<10^5 \text{ yr}^{-1}$ above our threshold of $S \sim 10^{-10}$ erg cm $^{-2}$ at 1 keV is shown on a recent log N -log S plot in Fig. 3; it is consistent with the turnover in the distribution inferred from the data at higher flux levels. Alternatively, we can use the result to set a limit on the value for E_x/E_{γ} as a function of E_{γ} ; for example, for $E_{\gamma} = 10^{37}$ erg, $E_x/E_{\gamma} \leq 0.03$. Expanding this survey by a factor of 10 to the entire Einstein database will clearly lead to important constraints on models for γ -ray bursts and on the space density of the systems in which they arise.

We have failed to exclude a celestial origin for the four nonstatistical events and so conclude with a brief discussion of the source population required to explain them.

The characteristic temperature of the burst spectra is $\sim 10^5$ K with no appreciable interstellar absorption (although, as a result of the poor statistics, $T \sim 10^6$ K and $N_H \sim 10^{21} \text{ cm}^{-2}$ are allowed spectral parameters). This low absorption leads to the con-

clusion that the events must originate within several hundred parsecs of the Sun. The integrated flux from the large burst was $\sim 2 \times 10^{-10}$ erg cm $^{-2}$, implying a rather modest energy of 2×10^{32} erg $D_{100}^{-2} \xi^{-1}$ where ξ is the fraction of the burst energy recorded by our instrument and D_{100} is the distance in units of 100 pc. The occurrence rate of $\sim 10^{-6} \text{ s}^{-1} \text{ deg}^{-2}$ is rather high, but the total time-averaged luminosity is negligible. For a source population with a scale height of ~ 250 pc and a uniform galactic distribution out to a radius of 12 kpc, we require an event rate of $2 \times 10^3 D_{100}^{-3} z_{250} \text{ s}^{-1}$ for the whole Galaxy or for $D_{100} = 2$ (our limit for these soft events), ~ 1 per star per decade; if the distance to the observed bursts is ~ 500 pc, the rate decreases by a factor of >100 . The population of inactive galactic neutron stars ($\sim 5 \times 10^8$ in number if a birthrate constant over the lifetime of the Galaxy of 1 per 40 yr is assumed) could, for example, be the source of the bursts if an event occurred on each star once every few days; the time-averaged energy required (roughly independent of the distance of the detected bursts) would be $\leq 10^{-3}$ of the rest mass energy of the material that such stars accrete as they move through the interstellar medium and $\sim 10^{-1}$ of the rotational energy loss rate for such objects.

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Orbital forcing of the inception of the Laurentide ice sheet?

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According to the astronomical theory of palaeoclimates, the glacial-interglacial oscillations of the Pleistocene have their origin in the insolation forcing caused by the orbital variations of the Earth¹. The potential response of the climatic system to the resulting insolation perturbations has been investigated mainly using zonally-averaged energy balance models (EBM)²⁻⁶. Atmospheric general circulation models (GCM) include many essential processes (such as the hydrological cycle) neglected in EBMs, but, because of their high computational cost, can be used only for 'snapshot' reconstructions of climate

at certain times in the past. We show here that the climatic transition at the end of the last interglacial is a particularly relevant period for GCM studies of the sensitivity of climate to the astronomical variations of insolation. We have used a low-resolution spectral GCM to simulate two annual cycles with the insolation conditions of 125,000 yr BP and 115,000 yr BP. For the 115-kyr simulation we find an annual mean cooling over Canada of more than 2 K and increased precipitation. We suggest that such a pattern of response to the insolation changes would favour the extension of permanent snow cover over the Labrador area and could have triggered formation of the Laurentide ice sheet.

The astronomical theory is probably the only theory of climatic change which can explain with any great accuracy the variations of the external forcing that have occurred during long periods of the past covering several million years⁷. Recent palaeoclimatic results have apparently confirmed the idea that orbital variations, which induce modifications of solar radiation distribution at the Earth's surface, have significantly influenced climate during the Pleistocene⁸ and may be the cause of the glaciations. One of the basic problems to be solved is how to explain quantitatively the mechanisms by which such changes in the zonal and seasonal distribution of insolation can lead to the accumulation or ablation of the enormous quantities of ice stored in continental ice sheets on the Northern Hemisphere.

The first step is to study the impact on the atmosphere alone of the insolation changes produced by the orbital variations. GCM studies are the best tools available at present for such sensitivity experiments. They have been effectively applied to the study of the climatic effects of changes of the solar constant⁹ and of the orbital parameters for 10,000 (ref. 10) and 9,000 (ref. 11) yr BP.

We performed two simulations of the general circulation of the atmosphere for a full annual cycle with the insolation conditions corresponding to 125,000 yr BP and 115,000 yr BP. These two experiments were chosen because: (1) They are the periods during the past 200,000 yr with the largest positive and negative deviations (from present conditions) of the July insolation in the Northern Hemisphere¹². (2) They mark approximately the beginning and end of isotopic substage 5e corresponding to the optimum of the last interglacial. 125 kyr corresponds to high sea-level stands (few metres higher than at present) recorded in marine terraces and coral reefs¹³, whereas 115 kyr marks the beginning of a glacial inception and the return to colder conditions¹⁴. During this whole period the North Atlantic polar front maintained a position like that of today^{15,16}, and there is evidence that sea-surface temperatures in the North Atlantic during this substage did not differ more than a few degrees from present-day temperatures (J. C. Duplessy, personal communication). Thus it seems realistic to perform both simulations at 125 kyr and 115 kyr using present sea-surface temperature and sea-ice limits¹⁷.

The model used for this experiment is a global GCM of the atmosphere based on the classical 'primitive equations' of atmospheric dynamics written in spherical coordinates. The vertical coordinate is the so called sigma coordinate¹⁸ p/p_s (p , pressure; p_s , surface pressure) which allows one to take into account the orography of the Earth's surface. The prognostic equations for horizontal velocity, temperature and water vapour mixing ratio are solved numerically by finite differences on 10

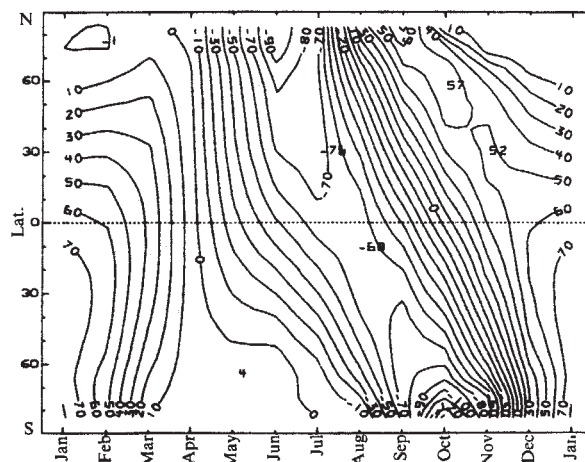


Fig. 1 Latitude-month distribution of the difference in monthly mean insolation (W m^{-2}) between 115 and 125 kyr BP.

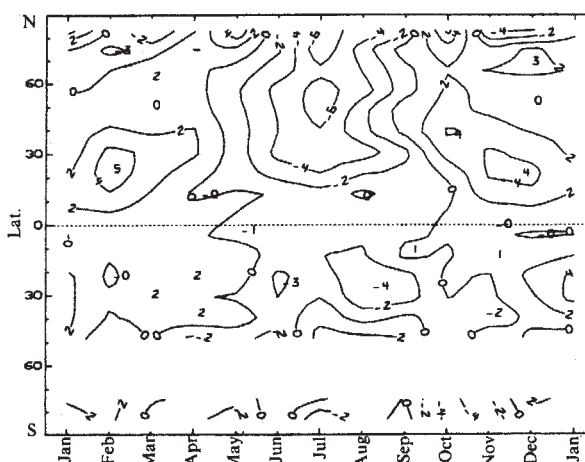


Fig. 2 Simulated latitude-month distribution of the difference (115 kyr minus 125 kyr simulations) of monthly mean temperature at the 850 mbar level (in $^{\circ}\text{C}$) zonally averaged over the continents.

vertical levels, and by truncated spectral decomposition in surface spherical harmonics Y_n^m of order m and degree n . Physical processes and nonlinear dynamical terms are computed on a latitude-longitude grid associated with the chosen truncation¹⁹. Transformations of the model variables from real grid point values to complex spectral components are achieved very efficiently by the Fast Fourier Transform method²⁰. The time integration of the prognostic equations is performed in the spectral domain with a semi-implicit scheme²¹. For this simulation we used a low-resolution truncation with $m \leq 10$, $n \leq 13$. The associated global grid has 20 latitude circles with 32 points equidistant in longitude. The time step for the numerical integration is 60 min.

This model has a parameterization scheme which includes the main physical processes. Radiation transfer is computed as a function of water vapour using the cooling to space and emissivity approximations²², which makes the scheme fast enough to include the diurnal variation of insolation. Cloudiness is computed diagnostically as a quadratic function of the relative humidity for three cloud layers²³ and its effect on radiative fluxes is specified by empirical formulae. The hydrological cycle is represented by the following processes: synoptic precipitation by large-scale saturation, moist-convective adjustment with a critical lapse rate function of the relative humidity of the layers, and deep convection by a modified Kuo's scheme²⁴. Surface fluxes of momentum, heat and water vapour are computed by the bulk aerodynamic formulae. The surface temperature over

Table 1 Values of the Earth's orbital elements adopted in the two simulations (present values are shown for comparison)

	125 kyr	115 kyr	Present
Obliquity (deg)	23.798	22.405	23.447
Eccentricity	0.040013	0.04142	0.01672
Longitude of the perihelion (deg)	307.14	110.9	102.0
(Corresponding time of year)	(24 July)	(14 January)	(3 January)

Fig. 3 Geographical distribution of the difference between the 115 kyr and 125 kyr simulations of the annual mean surface temperature ($^{\circ}\text{C}$).

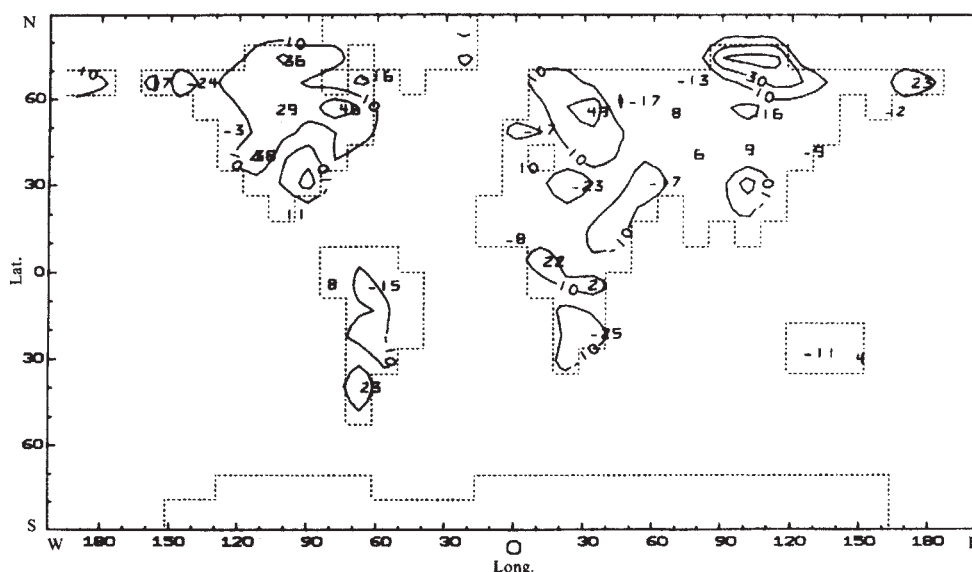
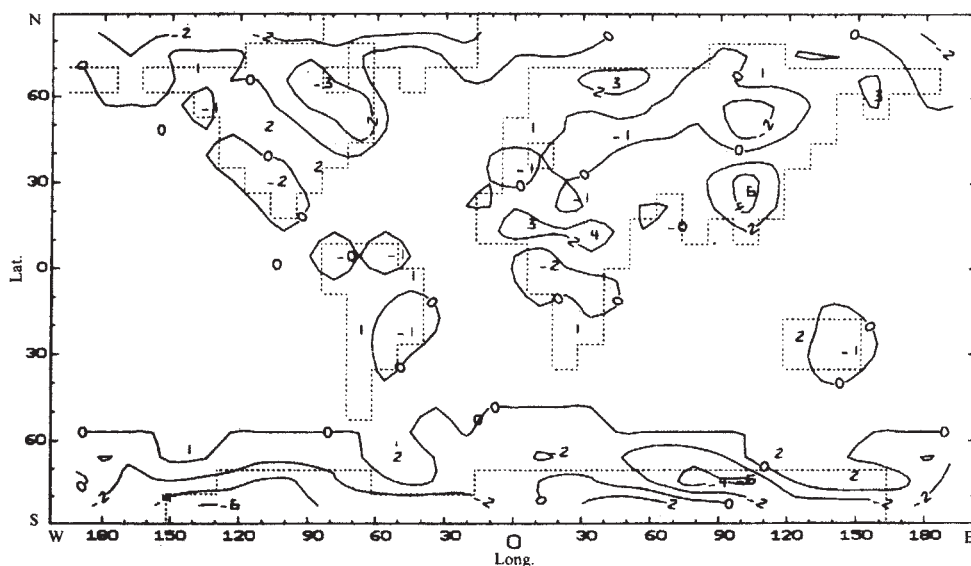


Fig. 4 Geographical distribution of the difference between the 115 kyr and 125 kyr simulations of the annual mean soil water content (kg m^{-2}).

land and ice is computed using a heat balance equation which takes into account heat conduction into the ground²⁵. Validation experiments performed with this model for January, July and several annual cycles have shown that it can simulate the main characteristics of the present climate.

The values of the orbital parameters adopted in our two experiments are displayed in Table 1 computed using a program of Berger²⁶. During the annual cycle the position of the Earth on its orbit is computed as a function of calendar date relative to a vernal equinox fixed conventionally at 21 March. The monthly mean insolation anomaly of 115 kyr relative to 125 kyr is displayed as a function of latitude and month in Fig. 1. This map, which can be viewed as the forcing function of the 115 kyr versus the 125 kyr experiment, shows a large insolation deficit of more than 70 W m^{-2} for Northern Hemisphere summer and a corresponding insolation surplus for Southern Hemisphere summer. Such a pattern is the direct consequence of the passing of the Earth at perihelion in January for 115 kyr and in July for 125 kyr with a large eccentricity.

As explained above, all other boundary conditions were identical in the two runs. Orography and land-sea distribution were fixed. Sea-surface temperature and sea-ice extent and thickness were interpolated each day during the integration from their present monthly climatological values¹⁷. The 125 kyr and 115 kyr integrations were both started at 1 December from the same initial situation. Statistics of the experiments were computed from the following January for a full annual cycle.

The difference between the 125 kyr and the 115 kyr simulations for the 850-mbar temperature zonally-averaged over the continents is shown in Fig. 2. Clearly, the seasonal cycle is strongly influenced by the insolation variation with cooler summers in middle and high latitudes for the Northern Hemisphere (up to 6°C in July at 50°N and near the Pole). The warming in winter has its maximum in lower latitudes so that the northern high latitudes (53° – 90°N) are on average 0.7°C colder at 115 kyr than at 125 kyr, during the year. This result is very significant for the Milankovitch theory as it demonstrates that the cold summers in the high latitudes of the Northern Hemisphere resulting from an insolation deficit when the Earth is at aphelion in July are not compensated by warmer winters.

If we look at the geographical distribution of the temperature difference between the two experiments, we see that in mean annual terms this cooling is not uniformly distributed over the high latitudes but has two well defined maxima, one over Siberia and the other over Canada (Fig. 3). The map of soil water content (Fig. 4), which reflects the balance of precipitation versus evaporation, shows an increase only over the latter area. Actually the maps of precipitation and evaporation (not shown) indicate that both increase over this area, but the increase in evaporation is insufficient to compensate for the increase in precipitation. The combination of cooling and increased precipitations is considered favourable to snow-cover expansion over the plateaus of Labrador and Baffin Island²⁷. According to the theories of 'instant glacierization', such snow-cover

expansion could be the factor that triggered the formation of the Laurentide ice sheet²⁸. However, the results of our experiment are only preliminary as with only 2 yr of simulation their statistical significance cannot be firmly established. A significance-testing formula²⁹ will be needed which requires several additional years of simulation, followed by a comparison with results from higher-resolution models. To explain the subsequent growth of the ice sheet, other factors not considered in this simulation, such as the albedo feedback and modifications of oceanic circulation^{30,31}, will have to be included in the model. Other simulations with more realistic surface conditions for the different phases of the ice sheet build-up will be necessary to analyse in detail the mechanisms responsible for the complete transition to glacial conditions.

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African monsoons, an immediate climate response to orbital insolation

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The Croll–Milankovitch astronomical theory of climate^{1–3} has received strong support from the evidence of a linear climatic forcing by obliquity and precession, although nonlinearity had to be assumed for eccentricity^{4,5}. Moreover, interglacials have appeared to be controlled by the orbital insolation⁶ although a phase shift of 6,000–5,000 yr is seen between an astronomical climate index and terrestrial climate indicators, dominated by the northern ice-sheet dynamics. Climate proxy data of low latitudes are less directly dependent on the ice sheets. Here it is shown that during the past 464,000 yr, African monsoons signalled by the East Mediterranean sapropels were heaviest always and only when a northern summer monsoon index, computed from the orbital variation of insolation, reached maximum values. This occurred during all the interglacials, but also twice during glacial periods. Thus tropical aridity is not the single climatic pattern of glacial periods. An immediate terrestrial climate response to orbital variations of insolation is established.

Heavy monsoons in Africa have been recorded by the black organic-rich layers called sapropels in the subsurface of the East Mediterranean Sea, a consequence of heavy discharge⁷ from the Nile River. I show here that they responded immediately to the variable distribution of northern-summer orbital insolation in time and space, described globally by the astronomical computation during the past 464,000 yr. Run-off from the inaccessible Northern Ethiopian Highlands orographic rains (May to September) produces the Nile River summer flood, 75–80% of the annual average discharge⁸. The annual progress of the mean monthly rainfall in Ethiopia⁹ shows that it belongs to the monsoonal Sudanian Zone, with southwesterly winds at 850 mbar at least from June to August¹⁰. The recent annual rainfall variability is similar in the Highlands, evaluated through the integrating mean Nile River discharge, and in the Sudanian Zone in West Africa. As in most of the tropics, a sudden change occurred in 1898¹¹, when the mean Nile regime decreased abruptly by 28% (ref. 8). Since 1898, the Nile flow^{8,11} and the Sudanian Zone^{12,13} have both undergone drought phases in the early 1910s, 1940s and late 1960s to mid-1970s, and relatively wetter phases in the 1930s and 1950s to early 1960s.

During the northern-summer monsoon in Africa^{13–15}, two synoptic elements are closely correlated with the rainfall variability, while the duration of the rainy season, normal during dry years, lengthens during wet years¹³. First, the amount of rain in the Sudan Zone diminishes when the seasonally migrating 'heat trough' is less marked, pressures being higher than average^{13–15}. It increases when the poleward excursion of the trough is larger^{13,16,17}.

Second, the equatorial westerlies, which straddle the Equator, seem to respond to the steepness of the meridional and longitudinal pressure gradients between the heat trough low pressure and the higher pressure near the cooler Equator^{14,15,18–23}.

In summer 1972, an extreme large-scale north tropical drought occurred in Africa, Central India, the Caribbean and Venezuela accompanied by a very strong wet El Niño in the south tropics^{12,16,17,24–28}. The African heat trough (ITCZ) was not marked, the Equator-to-ITCZ pressure gradient was small, the equatorial westerlies and their associated upper level easterly jets were weak while the low-level tropical easterlies were stronger^{16,17,28,29}. Zonally averaged atmospheric temperatures (surface to 100 mbar)³⁰ were globally below average in 1971, and until summer 1972 at 10–30°N (–0.6°C), while the Equator (–0.5°C in 1971) was already above average (+0.2°C) in summer 1972. This weakened the Equator (cold)–north tropics (warm) temperature gradient in summer 1972. Thus, the extreme drought was accompanied by lower tropical temperature and weaker Tropic–Equator gradient. The Southern Hemisphere temperature gradient was also decreased by above average South Pole temperatures from 1970 to 1975 (+0.4°C in 1972) and the south oceanic anticyclones were displaced southward¹⁷.

Since 1900, no extremely wet year has occurred in West Africa^{11,13}. In the less dry 1958^{12,13,26,27}, the global mean temperatures were above average in the northern subtropics (+0.6°C), below at the Equator (–0.7°C), producing a steeper Equator–Tropic gradient, and average at the South Pole³⁰.

Based on the previous analysis, the following synoptic situation is proposed for presently unknown very heavy African monsoon: (1) A well marked heat trough, with very low pressures, reaching the Tropic (23°27'N) in summer. (2) Strong Equator-to-Tropic baroclinicity. The Southern Hemisphere winter baroclinicity, (20–70°S) reflects the strength of the southern winter trade-winds¹⁰.

To reconstruct the African monsoons of the Quaternary, this prescribed meteorology will be formulated by using the boundary radiative values of orbital insolation during the caloric northern summer half-year².

The insolation at the north Tropic I_T reflects the intensity of the heat trough. The latitude of the Tropic shifts by 2°41'

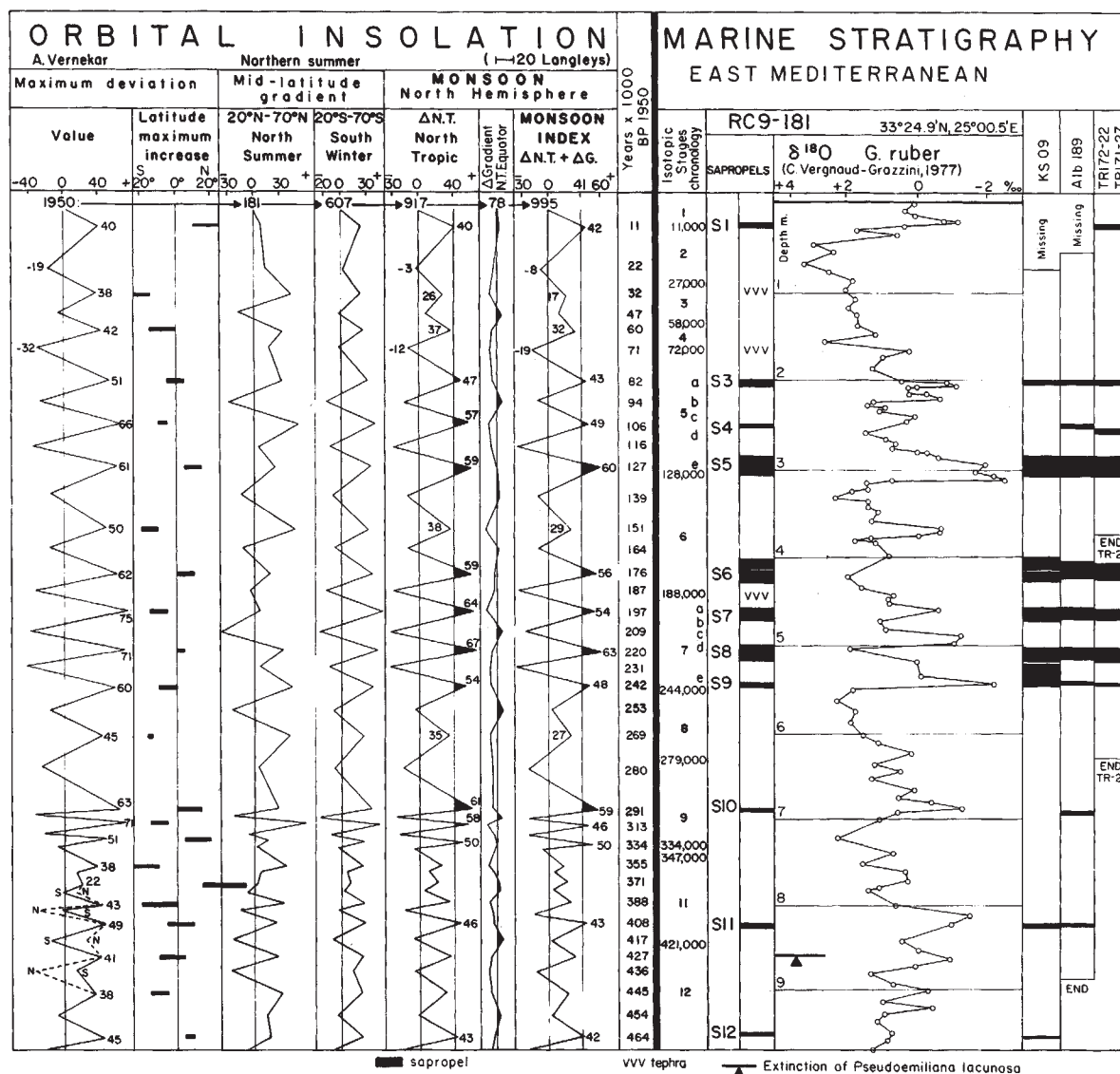


Fig. 1 Correlation of the orbital insolation monsoon index (caloric half year northern summer) with deep-sea sapropels in the East Mediterranean—insolation in langley (cal cm⁻²) day⁻¹ in Δ AD 1950. Absolute value of AD 1950 insolation on top line, ref. 2. Isotopic stages chronostratigraphy, ref. 32. Core RC9-181; 33°25' N, 25°00' E; 2,286 m ref. 31. Core KS-09; 35°09' N, 20°09' E; 2,800 m ref. 34. Core Alb. 189; 33°54' N, 28°29' E; 2,604 m, ref. 35. Core Tr 172-22; 35°19' N, 29°01' E; 3,150 m, ref. 36. Core Tr 172-27; 33°50' N, 25°59' E; 2,680 m, ref. 36. Vertical scale for orbital insolation and isotopic chronostratigraphy based on RC9-181 core depth.

according to the Earth's axis obliquity, with a 41,000-yr period¹⁻³.

The gradient of insolation $I_T - I_E$ between the north Tropic and the Equator reflects the baroclinicity between these latitudes.

An orbital insolation monsoon index M at time t is established:

$$M' = I_T' + (I_T' - I_E') = 2I_T' - I_E'$$

The variation of M for the past 464,000 yr is shown in Fig. 1. The astronomical time scale is adjusted on the $\delta^{18}\text{O}$ chronostratigraphy of core RC9-181 in Fig. 1. The Southern Hemisphere winter baroclinicity is described by the 20°–70° S insolation gradient:

$$G_s = I_{20} - I_{70}$$

The insolation values appear as their deviation from the AD 1950 absolute values, shown on the upper line. The periods of highest insolation of the northern summer display the highest monsoon index M , as well as the largest Southern Hemisphere winter gradients.

The East Mediterranean sapropel and $\delta^{18}\text{O}$ stratigraphy of core RC9-181 (ref. 31) is shown in Fig. 1. The chronostratigraphy of the $\delta^{18}\text{O}$ stage boundaries³² establishes the correlation of the Mediterranean marine record with the monsoon index. The extinction of the coccolith *Pseudoemiliana lacunosa*, observed worldwide within stage 12 (ref. 33), has been recognized below sapropel 11 (ref. 31). Reexamination of the core showed that sapropel 2 does not exist (F. McCoy, personal communication).

A sapropel was formed every time the increasing northern summer insolation produced an index M above the threshold value of 41, and no sapropel occurred outside of these periods. The origin of the only exception, 'missing' sapropels in stage 9, has to be investigated. Four other cores with $\delta^{18}\text{O}$ curve are also shown³⁴⁻³⁶. The epipelagic $\delta^{18}\text{O}$ depletion events of the Nile floods appear when the sampling interval is very dense⁷.

Two physical parameters combine to produce threshold M : the magnitude and the latitude of the insolation maxima, seen in the first two columns of Fig. 1. These maxima have to be higher in the Southern than in the Northern Hemisphere to reach threshold M . At 11,000 yr BP, the maximum was the

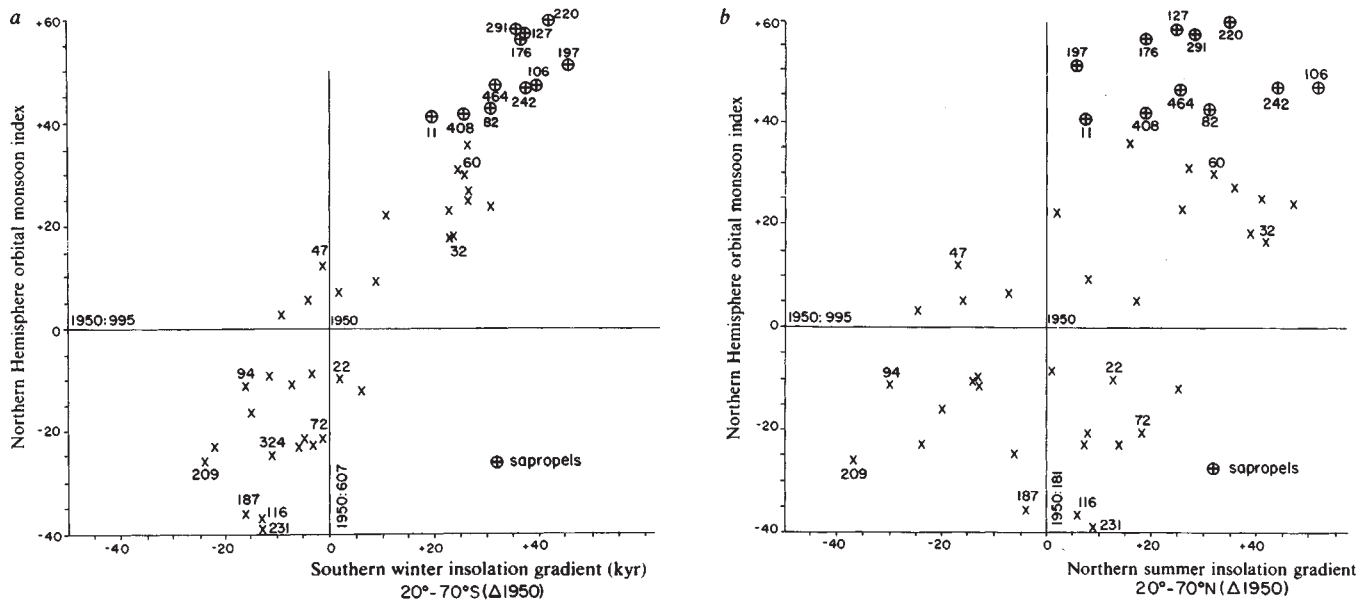


Fig. 2 Northern Hemisphere monsoon index, sapropel formation and *a*, southern winter; *b*, northern summer mid-latitude insolation gradient reflecting the strength of *a*, southern winter *b*, northern summer trade-winds. Insolation as in Fig. 1.

smallest which triggered a sapropel, but it was the furthest north (10–25° N).

The mid-latitude insolation gradient, reflecting trade-wind strength, is controlled by the 20° lat. insolation, since the largest orbital insolation variations occur in the tropics. As seen in Fig. 2, high *M* and sapropel formation correlate well with the largest insolation gradients of the southern winter, supporting the 1972 drought analysis and Kraus's hypothesis¹², but not with those of the northern summer.

Corresponding to highest *M*, sapropels deposited most frequently during the warming phases of interglacials^{31,34–36}. Northern tropical rainfall in all longitudes was heaviest from 12,500 to 8,000 yr BP (ref. 7), particularly during the Indian monsoon^{37–39}, successfully simulated at 9,000 yr BP (refs 40, 41). During the 127,000-yr BP Eemian insolation peak, the Indian monsoon also increased³⁷, and lakes appeared in Libya around 130,000 yr BP (ref. 42). The sapropel pollen record seen in Fig. 3 shows the warm, sub-humid character of the Mediterranean area vegetation during these interglacial sapropels, with high values for trees (*Quercus* and *Pinus*), low for steppe (*Artemisia*).

However, the thick and widespread sapropels S6 and S8 formed during periods with large ice sheets: glacial stage 6, and cold interstadial 7d within interglacial stage 7. Their planktonic fauna was cold^{31,34}, and their pollen record points to a continental dry Mediterranean climate, with high values for steppe, low for trees. Precisely during these two periods, *M* was among the highest ever at 176,000 yr BP and 220,000 yr BP, with the insolation peaks centred at 0–10° and 0–5° N lat. Actual trade winds were the strongest ever, enlarged by the tropical warming and the polar ice-sheets cooling. At 220,000 yr BP, highest *M* followed very abruptly a record low at 231,000 yr BP. At 60° N, the very low June-to-August insolation between 230,000 and 225,000 yr BP (ref. 3), resulted in the voluminous ice-sheet depicted by the previously described^{4,43}, brief but severe isotopic enrichment of stage 7d.

Thus African tropical aridity during northern glacial periods, as seen at 18,000 yr BP, is not an exclusive climatic pattern. Large northern ice sheets have been coeval with very wet African northern tropics, suggesting that ice-sheet global cooling alone is not able to produce tropical aridity. High local tropical insolation appears therefore as the foremost climatic parameter correlated with heavy African monsoon, and the difference between the tropical aridity around 18,000 yr BP and

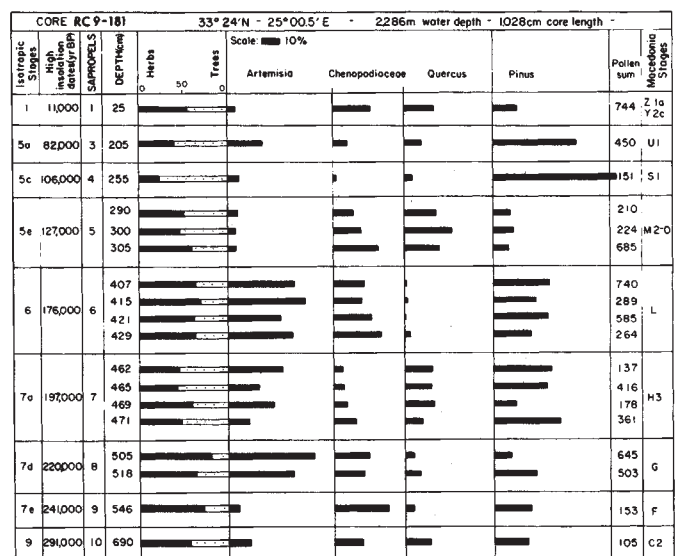


Fig. 3 Elements of pollen spectra of sapropels in Mediterranean core RC9-181. Sapropel numeration as in Fig. 1. Macedonia stages from refs 59, 60.

the 176,000 and 220,000 yr BP heavy monsoons is prominently the tropical insolation.

The north-tropical lowest insolation of 22,000 yr BP, which was only –3 langley at 25° N, triggered the arid period which lasted until 13,000 yr BP and suggests feed-back processes perpetuating the drought through increased albedo^{12,44–46}.

During the last glacial, *M* was always higher than presently from 25,000 to 66,000 yr BP, with minor peaks at 32,000 and 60,000 yr BP. Correspondingly, a wet phase is recorded around and below 30,000 yr BP by sapropels near the Nile mouth⁴⁷, around 30,000 yr BP in sub-Saharan Africa^{48–52}, between 40,000 and 20,000 yr BP in Lake Tchad⁵³, between 30,000 and 21,000 yr BP in Ethiopian Rift Lakes^{54,55}, around 36,000 and from 30,000 to 21,000 yr BP in south-west Arabia^{56,57}.

The strong correlation in the tropics between warm periods and heavy rainfall suggests that in the geological past during

such periods, whatever their cause, heavy precipitation on the continents extended stratified estuarine circulation to continental shelves and oceanic basins, and produced marine organic-rich sediments similar to the Quaternary Mediterranean sapropels, which eventually became petroleum and phosphorite source beds. The same process accounts for the origin of stratabound ores associated with organic-rich black shales.

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Radiocaesium from Sellafield effluents in Greenland waters

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Since the middle of the 1970s, discharges of radiocaesium to the Irish Sea from the British Nuclear Fuels Ltd Sellafield (formerly Windscale) installation in Cumbria have increased the concentrations of ^{137}Cs and ^{134}Cs in the northern North Atlantic. In coastal British waters^{1,2} enhanced concentrations have also been seen in the North Sea^{3–5}, the Danish Straits and western part of the Baltic Sea^{4,5}, the Norwegian coastal current^{6,7}, and the Barent and Greenland Seas⁷. We report here that radiocaesium from Sellafield is now detectable in the East Greenland polar current. The transit time from Sellafield is estimated at 6–8 yr. The concentrations in the polar current are approximately one-thousandth of those found in the North Channel by the outlet from the Irish Sea.

In August 1982 groups from Risø National Laboratory and Lund University collected 200-l samples of the surface seawater along the Greenland east coast (see Table 1) using a pump from M/S *Nella Dan*. The radiocaesium was contained in precipitates of 100 g AMP (ammonium molybdophosphate)⁸ taken on board and later measured by Ge(Li) spectroscopy for ^{137}Cs . To determine ^{134}Cs it was necessary to concentrate the activity. After being dissolved in NaOH the radiocaesium in 0.5 kg AMP aliquots (representing 1 m³ of water) was reprecipitated as 0.5 g Cs₂PtCl₆ with a 90% recovery of activity. This procedure improved the measuring geometry and removed ^{228}Ra and ^{228}Ac (γ photon: 794.9 keV), which disturbed the γ measurement of ^{134}Cs (γ photons: 604.7 keV and 795.8 keV) when performed directly on the AMP. The removal of ^{228}Ac was so efficient that both photo peaks of ^{134}Cs gave the same results, and the high resolving power of our Ge(Li) spectrometer meant that it was not necessary to correct for interference from the 609.4 keV peak of ^{214}Bi . The procedure applied had a lower limit of detection for ^{134}Cs of 0.01 Bq m⁻³.

The mean ^{137}Cs concentration at the five western stations, 5.43 ± 0.08 (± 1 s.e.m.) Bq m⁻³, was significantly ($P \sim 99\%$) lower than that of the five eastern ones, 6.70 ± 0.38 Bq m⁻³. The ^{134}Cs concentration was 0.043 Bq m⁻³ at the eastern and 0.012 Bq m⁻³ at the western stations.

Fallout from nuclear-weapons testing in the atmosphere is the main source of ^{137}Cs in the oceans. To determine the 'non-Sellafield' contribution of ^{137}Cs in the samples we utilized our measurements of surface seawater collected at Danmarkshavn (76°49'N, 18°36'W) and Angmagssalik (65°35'N, 37°52'W) during 1978–80. Östlund¹⁰ has found that 'the average age of the freshwater component is 11 ± 1 yr from its first appearance at the shelf to its emergence from the basin as a constituent of the modified Atlantic (Arctic) water in the East Greenland current'. Both Danmarkshavn and Angmagssalik are situated in the polar current respectively north and south of our sampling stations. This residence time of ~ 11 yr is compatible with our own ^{90}Sr and ^{137}Cs observations in surface seawater collected annually at Danmarkshavn and Angmagssalik since 1963.

The time constant λ for the decrease of ^{137}Cs in the polar current is the sum of the radioactive decay constant for ^{137}Cs , λ_{Cs} ($= \ln 2/30.5 \text{ yr}^{-1}$), and the time constant for the freshwater component in the Arctic Ocean is λ_{AO} ($= 1/11 \text{ yr}^{-1}$).

$$\text{Hence } \lambda = 0.023 + 0.091 = 0.114 \text{ yr}^{-1}$$

which corresponds to an effective half life of ^{137}Cs in the polar current of $\ln 2/0.114 = 6.1 \text{ yr}$.

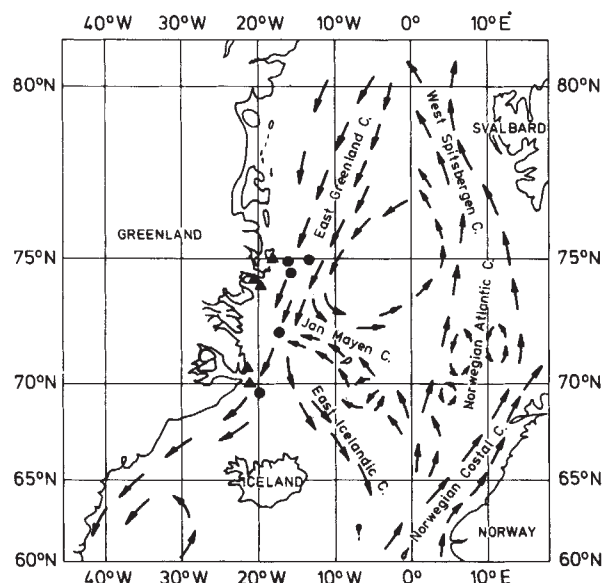


Fig. 1 Circulation of the upper layers of the Greenland and Norwegian Seas. Δ , Western stations; $\bullet$, eastern stations.

We then calculated what the 1978–80 concentrations of ^{137}Cs would have been at these stations in 1982, if each year's levels had decayed with a half life of 6.1 yr. The mean of the six such decay-corrected determinations was 5.0 ± 0.25 (1 s.e.m.) $\text{Bq } ^{137}\text{Cs m}^{-3}$. We found no indication of an increase in the calculated concentrations with time, and we assume therefore that there have been no significant contributions from Sellafield in the East Greenland polar current before 1981–82. Unfortunately, we had obtained no seawater samples from Greenland during 1981 and could not see whether or not Sellafield effluent showed up in that year. In August 1981, H. D. Livingston (personal communication) found in surface water at $63^\circ 07' \text{N}$, $34^\circ 32' \text{W}$ $5.78 \text{ Bq } ^{137}\text{Cs m}^{-3}$ and $0.06 \text{ Bq } ^{134}\text{Cs m}^{-3}$, which may indicate the presence of Sellafield effluent in 1981 in the polar current. Livingston's ^{134}Cs result corrected for decay agrees with our measurement at the eastern stations.

Our best guess is that the increased discharges of radio-caesium from Sellafield, which began in 1974, appeared at East Greenland in 1981 and that the 1982 levels thus represent 1975 discharges. If this is true, we would expect a $^{134}\text{Cs}/^{137}\text{Cs}$ ratio in the radio-caesium from the discharge data, attributed to Sellafield in the polar current, of 0.028 in 1982. At the eastern five stations the measured ratio becomes $0.043(6.7-5.0)^{-1} = 0.025 \pm 0.005$, that is in agreement with the 1975 discharge. However, this measured ratio will also be compatible with all the other years in the 1974–78 period with increased ^{137}Cs discharges ($4-5 \text{ PBq yr}^{-1}$). If the measured concentrations in 1982 were from the last years of enhanced discharges,

that is 1977–78, we would have expected to have seen an increase in ^{137}Cs levels at Danmarkshavn and Angmagssalik in 1978–79 due to the appearance of the 1974 discharge. As this was not the case, however, we conclude that the enhanced ^{137}Cs levels in 1982 at the five eastern stations in the polar current were due to Sellafield discharges in 1975 (± 1 yr).

The eastern stations are, however, located close to the polar front, where Atlantic-derived water mixes with the polar water. Swift and Aagaard¹¹ showed that the tritium concentrations decrease rapidly when moving from west to east in this region. Tritium may be used as a tracer for fallout. We consequently measured the tritium concentrations at the western and eastern locations and found 4.3 ± 0.44 and $3.5 \pm 0.37 \text{ kBq } ^3\text{H m}^{-3}$ respectively (± 1 s.e.m.). Although our tritium measurements show a tendency to decrease from west to east, the difference between our western and eastern stations in the East Greenland current was not statistically significant. We shall therefore keep to our hypothesis that the estimated fallout background of $5 \text{ Bq } ^{137}\text{Cs m}^{-3}$ is applicable both to the western and to the eastern locations.

As regards the five western stations in Table 1, a possible Sellafield contribution is calculated to be $5.43-5.0 = 0.43 \pm 0.26 \text{ Bq } ^{137}\text{Cs m}^{-3}$. This is a factor of about four lower than the contribution at the eastern stations. The determination of ^{134}Cs at these stations is encumbered with a large error due to the low concentration, and the ^{134}Cs concentration was not significantly different from zero activity. The $^{134}\text{Cs}/^{137}\text{Cs}$ ratio in possible Sellafield effluent at the western stations becomes 0.03 ± 0.02 . It is thus not possible to use this ratio to identify the discharge year. We assume tentatively that the western stations in the polar current may contain Sellafield ^{137}Cs from discharges in the first part of the 1970s. This requires a longer path of transmission from Sellafield to the western part of the East Greenland current than to the eastern part. Furthermore, the longer transit path does not necessarily mean a greater dilution of the activity.

We may imagine two routes from Sellafield to the East Greenland polar current (see Fig. 1). One is rather fast, which after leaving the Norwegian coastal current joins the Norwegian Atlantic current. Between 75° and 80°N , west of Svalbard, it turns westward and enters the eastern edge of the East Greenland current. Another slower route follows the Norwegian Atlantic current, enters the West Spitzbergen current and joins the polar current in the Arctic Ocean somewhere north of Svalbard. Preliminary results from British (D. F. Jefferies, personal communication) and German (H. Kautsky, personal communication) cruises to the Barents, Norwegian, and Greenland Seas in 1981 and 1982 seem compatible with such a transit pattern. As the latter route follows the main currents to a larger extent than the former, the dilution factors may be similar for both routes.

Jefferies *et al.*¹ have estimated the transit time from Sellafield to the North Channel as ~ 1 yr. Kautsky¹² has shown that the

Table 1 Radiocaesium in surface seawater collected in the East Greenland polar current

Position (N)	Position (W)	Sampling date (August 1982)	^{137}Cs (Bq m^{-3})	^{134}Cs (Bq m^{-3})	Salinity	Temperature ($^\circ\text{C}$)
Western stations						
$70^\circ 01.8'$	$21^\circ 07.9'$	5	5.56 ± 0.22		29.5	2
$70^\circ 34.9'$	$21^\circ 16.5'$	7	5.21 ± 0.16		30	-0.3
$73^\circ 59.5'$	$19^\circ 49.4'$	8	5.27 ± 0.21	0.012 ± 0.005	—	1
$74^\circ 18.6'$	$20^\circ 14.3'$	9	5.46 ± 0.22		30	0.5
$74^\circ 58.9'$	$18^\circ 22.4'$	13	5.67 ± 0.23		28	1.5
Eastern stations						
$74^\circ 55.3'$	$13^\circ 36.4'$	17	6.86 ± 0.21		29	-1
$74^\circ 42.3'$	$16^\circ 00.4'$	19	6.08 ± 0.18		29	-1
$74^\circ 28.7'$	$15^\circ 52.1'$	20	6.38 ± 0.26	0.043 ± 0.006	30	-1.5
$72^\circ 12.7'$	$17^\circ 05.2'$	21	8.09 ± 0.16		30	1
$69^\circ 27.8'$	$20^\circ 03.1'$	25	6.07 ± 0.24		29.5	-0.5

The error terms due to counting statistics are 1 s.d.

effluent from Sellafield passes the Central North Sea before it enters the Norwegian coastal current on its way northward. H. D. Livingston (personal communication) has measured the $^{134}\text{Cs}/^{137}\text{Cs}$ ratios in the Norwegian coastal current during 1976–81. His measurements suggest a transit time from Sellafield to this current of 3–4 yr. We estimate that the transit time to East Greenland from the North Channel by the above mentioned fast route is 5–7 yr. If the radiocaesium concentrations in 1975–77 in the North Channel corrected for decay to 1982 are compared with those attributed to Sellafield at the eastern five stations in the polar current, we observe a dilution factor of 10^3 . The dilution factor between radiocaesium levels in North Sea and polar current water is estimated at 10^2 and the transit time is ~ 2 yr shorter than from the North Channel⁶.

Our results suggest that effluents from Sellafield may be used as a tracer for waterborne pollution in the North Sea, which is a main recipient for northern and western Europe. Pollution from the North Sea enters coastal Greenland waters 3–5 yr later in concentrations of the order of 1% of those found in the North Sea. The estimated individual doses from present concentrations of radiocaesium from Sellafield in Greenland waters are of the order of 10^{-7} Sv yr⁻¹, which is extremely low.

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Pb, Sr and Nd isotopic evidence for sources of southern African Cretaceous kimberlites

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Previously reported initial $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios of non-micaceous southern African Cretaceous kimberlites indicate that their mantle source rocks are undifferentiated to slightly depleted relative to the bulk Earth^{1–6}. Anomalous radiogenic Pb isotopic ratios of these kimberlites, on the other hand, are similar to those of oceanic island basalts and indicate that the source regions have had high U/Pb ratios⁷. Some micaceous kimberlites have a different Sr and Pb isotopic character, reflecting different source regions characterized by higher Rb/Sr, but lower U/Pb ratios^{1,2,7}. Nd isotopic analyses have not been reported for the micaceous kimberlites. I present here new whole-rock Pb, Sr and Nd isotopic analyses of examples of both types of kimberlite which, in conjunction with previously published data, confirm the existence of both depleted and enriched upper mantle sources for southern African Cretaceous kimberlites.

Southern African late Jurassic and Cretaceous kimberlite occurrences can be subdivided into two groups on the bases of their emplacement ages and mineralogical (and therefore geochemical) character. Members of the first group (Group I) have emplacement ages of 80–114 Myr (refs 8–11) (Table 1).

Group I kimberlites are predominantly non-micaceous at any given locality, in that late-crystallizing phlogopite is absent or comprises only a small proportion of the groundmass constituents except in minor aphanitic to microporphyritic hypabyssal varieties. In contrast, Group II kimberlites contain phlogopite as a dominant groundmass mineral, and have older emplacement ages ranging from 114 to 150 Myr (refs 8, 12) (Table 1). Group I and Group II kimberlites correspond to the basaltic and micaceous varieties of Dawson¹³. Major variations in initial Sr, Pb and Nd isotopic compositions are correlative with these two groups.

Analytical techniques for Sr and Pb are essentially the same as reported previously for kimberlite work^{2,7}. Sm and Nd were separated by mixed-solvent anion exchange modified from the technique described by Hooker *et al.*¹⁴ after initial separation of rare-earth elements (REE) by conventional cation exchange as used for Sr separation. Nd³⁺ was analysed using a triple filament configuration on a Micromass 30 mass spectrometer. Because a ^{146}Nd -enriched spike is used, $^{143}\text{Nd}/^{144}\text{Nd}$ ratios were corrected for fractionation using $^{145}\text{Nd}/^{144}\text{Nd} = 0.34844$ obtained by normalizing atomic abundances reported by Weast and Selby¹⁵ against abundances reported for NdO⁺ measurements¹⁶. Analysis of BCR-1 gives $^{143}\text{Nd}/^{144}\text{Nd} = 0.51270 \pm 2$ (1 s.e.m. of four analyses). Analytical results are given in Tables 1 and 2.

Some of the first whole-rock isotopic data for kimberlites^{1,2} indicated that fresh basaltic (Group I) and micaceous (Group II) varieties have initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of about 0.704 and 0.708 respectively, and the present work has verified this relationship. Initial $^{87}\text{Sr}/^{86}\text{Sr}$ compositions of Group I kimberlites range from 0.7033 to 0.7049. Group II kimberlites have substantially higher and more variable isotopic compositions from about 0.7074 to 0.7109 (Fig. 1). The relatively few initial Sr ratios determined at this Institute which fall between the ranges defined by these two groups are from altered or visibly contaminated samples.

The first reported initial Pb isotopic compositions of southern African Cretaceous kimberlites⁷ are similar to those of modern oceanic island basalts, with radiogenic and variable $^{206}\text{Pb}/^{204}\text{Pb}$. Exceptions are the Bellsbank and Finsch kimberlites, which have relatively unradiogenic Pb isotopic compositions more primitive than mid-ocean ridge basalts (MORB). Kramers⁷ interpreted the overall linear array as a 1,575-Myr 'isochron' indicative of the minimum time span that the source rocks of the various kimberlites have had separate histories. It is now apparent that the Group I kimberlites have distinctly radiogenic and variable Pb isotopic compositions ($^{206}\text{Pb}/^{204}\text{Pb} = 18.45\text{--}20.05$) relative to Group II occurrences ($^{206}\text{Pb}/^{204}\text{Pb} = 17.2\text{--}17.7$, Fig. 1), and higher U/Pb ratios due to lower Pb contents (Tables 1 and 2). Addition of the new analyses results in considerably more data scatter (Fig. 1). Jagersfontein, Jwaneng and two new samples from Monastery and Benfontein sill have considerably lower $^{207}\text{Pb}/^{204}\text{Pb}$ ratios relative to the previous data array⁷. This is not believed to be caused by analytical bias as analyses of other Group I kimberlites plot within the previous array, and the Pb isotopic composition of the Group II Finsch kimberlite is in close agreement with previous determinations⁷. The increased scatter renders interpretation of the data array as having age significance more tenuous, although the overall slope is essentially equivalent to that determined by Kramers. As is the case for the Sr systematics, there is a distinct compositional gap between Group I and Group II kimberlites (Fig. 1). Pb and Sr isotopic compositions are inversely correlated between the two groups (Fig. 1).

Published $^{143}\text{Nd}/^{144}\text{Nd}$ ratios of southern African Cretaceous kimberlites indicate that, with one exception¹⁷, they are derived from nearly undifferentiated^{4–6} or slightly depleted^{3,17} sources with respect to a chondritic reservoir. Initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios of Group I kimberlites reported here vary within relatively narrow limits of 0.51271–0.51277 (relative to $^{143}\text{Nd}/^{144}\text{Nd} = 0.51270$ in BCR-1) and are in close agreement with results of Kramers *et al.*³ except for the Frank Smith kimberlite with

Table 1 Initial isotopic ratios and parent/daughter atomic ratios for Group I and Group II kimberlites

Sample	Kimberlite	Age (Myr)	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{238}\text{U}/^{204}\text{Pb}$	$(^{87}\text{Sr}/^{86}\text{Sr})_0$	$(^{143}\text{Nd}/^{144}\text{Nd})_0$	$(^{206}\text{Pb}/^{204}\text{Pb})_0$	$(^{207}\text{Pb}/^{204}\text{Pb})_0$
Group I									
JAG-K9*	1	86 ¹¹	1.52±	0.085	510±	0.7044±	0.51275	19.27±	15.57±
JAG-K4†	1		0.400	—	17.8	0.7053	—	18.94	15.60
JAG-K7†	1		0.056	—	25.4	0.7055	—	18.94	15.52
JAG-K10	1		—	—	37.6	—	—	19.00	15.54
Uin 1	2	100	0.044	0.096	52.1	0.7041	0.51271	19.04	15.69
Uin 2†	2		0.110±	—	61.3	0.7044±	—	18.68	15.72
RJ53 + 54K	3	90 ⁸⁻¹⁰	0.371	0.098	34.7	0.7033	0.51276	19.09±	15.53±
BFK-1	4	—	0.038	0.087	17.2	0.7048	0.51272	18.67	15.52
K7/10 B	5	—	0.298±	0.090	29.3	0.7037±	0.51277	19.00±	15.57
FS-K1	6	114	0.107	0.090	46.1	0.7044	0.51251	19.03	15.65
K61/35*	6		0.436±	—	48.3	0.7049±	—	19.05±	15.64±
Group II									
NE-K2†	7	127	0.365	—	9.64	0.7076	—	17.21	15.48
NE-K4	7		0.300	—	10.9	0.7075	—	17.26	15.47
NE-K6	7		0.354	—	7.97	0.7076	—	17.22	15.47
NE-K10	7		0.349	0.076	6.77	0.7074	0.51208±	17.22±	15.47±
K64/55	8	120	0.226	0.065	8.89	0.7075	0.51214	17.48±	15.50±
Bsb1	8		0.559	—	—	0.7090	—	—	—
NS	9	114	0.224	—	11.2	0.7074	—	17.47	15.47
K56/15	9		0.140	0.062	—	0.7077	0.51215	—	—
Finsch B*	10	118	0.668±	0.087	19.0	0.7087±	0.51227	17.63	15.52
Male 14/1	11	145 ^{8,12}	0.324±	0.086	15.1	0.7099	0.51206	17.64±	15.51±
Main 14/3	11		0.652	—	—	0.7080	—	—	—
Main 14/6	11		0.611	—	—	0.7088	—	—	—
Main 15/0†	11		0.741	—	10.4	0.7094	—	17.63	15.62
Main 15/1	11		0.786	—	—	0.7109	—	—	—
Klip 3	12	~150	0.244	—	8.36	0.7077	—	17.39	15.49

Kimberlites: 1, Jagersfontein (29°46' S, 25°28' E); 2, Uintjesberg (30°51' S, 22°32' E); 3, Monastery (28°49' S, 27°25' E); 4, Benfontein sill (28°48' S, 24°50' E); 5, Jwaneng satellite pipe (24°33' S, 24°41' E); 6, Frank Smith (28°14' S, 24°30' E); 7, New Elands (28°32' S, 25°28' E); 8, Bellsbank (28°05' S, 24°24' E); 9, Newlands (28°19' S, 24°25' E); 10, Finsch (28°16' S, 23°06' E); 11, Swartruggens (25°35' S, 26°39' E); 12, Klipfontein (28°23' S, 24°08' E). Emplacement ages: superscripts refer to reference, other ages from Rb-Sr on phlogopite (to be presented elsewhere). Reliable ages are not available for Benfontein or Jwaneng; ages are taken as 90 Myr. The reliability of the age for Klipfontein is questionable: Klipfontein is not a kimberlite sensu stricto in that it contains minor amounts of groundmass feldspathoid. Analytical uncertainties on $^{87}\text{Rb}/^{86}\text{Sr}$, $^{147}\text{Sm}/^{144}\text{Nd}$ and $^{238}\text{U}/^{204}\text{Pb}$ are 1.6, 0.3 and 2.4% respectively. $^{238}\text{U}/^{235}\text{U} = 137.88$. Decay constants: $^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$; $^{147}\text{Sm} = 6.54 \times 10^{-12} \text{ yr}^{-1}$; $^{238}\text{U} = 1.55125 \times 10^{-10} \text{ yr}^{-1}$; $^{235}\text{U} = 9.8485 \times 10^{-10} \text{ yr}^{-1}$.

* Aphanitic to microporphyritic hypabyssal kimberlite.

† Slightly altered or contaminated samples.

‡ Average of duplicate concentration and/or isotopic ratio analyses.

Table 2 Element concentrations and measured isotopic ratios for Group I and Group II kimberlites

Sample	Rb	Sr	Sm	Nd	U	Pb	$^{87}\text{Sr}/^{144}\text{Sr}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$
Group I											
JAG-K9*	153.3±	291.4±	68.55	509.0	15.1±	2.34±	0.70619±7±	0.51280±2	26.03±2±	15.89±1±	48.48±4±
JAG-K4†	34.2	247.1	—	—	1.23	4.46	0.70582±3	—	19.18±2	15.61±2	38.99±3
JAG-K7†	21.8	1,122	—	—	8.55	21.8	0.70553±17	—	19.28±1	15.54±1	39.20±3
JAG-K10	—	—	—	—	2.28	3.93	—	—	19.50±3	15.56±2	39.24±5
Uin 1	14.40	953	37.75	284.4	7.68	9.76	0.70418±5	0.51278±3	19.85±1	15.73±1	40.06±2
Uin 2†	49.0±	1,284±	—	—	8.21	8.85	0.70457±7±	—	19.64±2	15.77±2	40.12±6
RJ53 + 54K	113.4	881	13.12	84.07	3.28	6.18	0.70377±11	0.51282±2	19.58±2±	15.57±2±	39.43±5±
BFK-1	8.99	691	14.39	104.8	3.06	11.4	0.70489±5	0.51279±2	18.915±3	15.530±3	38.75±1
K7/10 B	31.4±	304.0±	5.81	40.80	2.48	5.54	0.70412±10±	0.51282±2	19.42±2±	15.59±1±	40.01±4±
FS-K1	29.46	797	17.60	125.6	5.44	7.79	0.70461±8	0.51257±3	19.84±1	15.69±1	39.90±2
K61/35*	156.5±	1,036 ±	—	—	6.98	9.62	0.70564±8±	—	19.91±3±	15.68±3±	40.77±4±
Group II											
NE-K2†	153.1	1,225	—	—	6.17	39.6	0.70824±4	—	17.41±1	15.49±1	37.90±2
NE-K4	155.9	1,519	—	—	5.25	29.9	0.70799±7	—	17.479±4	15.482±4	37.88±1
NE-K6	170.3	1,390	—	—	3.95	30.7	0.70819±11	—	17.383±6	15.475±5	37.86±1
NE-K10	177.7	1,471	18.53	154.8	4.47±	41.2±	0.70807±4	0.51214±2±	17.36±1±	15.48±1±	37.74±2±
K64/55	112.6	1,438	18.69	182.1	4.63	32.5	0.70791±14	0.51219±3	7.647±6±	15.510±6±	38.06±2±
Bsb1	120.7	628	—	—	—	—	0.70990±8	—	—	—	—
NS	73.5	947	—	—	5.8	31.8	0.70773±11	—	17.664±2	15.476±2	37.96±2
K56/15	39.6	777	9.99	101.9	—	—	0.70795±8	0.51219±3	—	—	—
Finsch B*	156.7±	678±	6.68	48.22	2.82	9.29	0.70987±6±	0.51234±2	17.98±3	15.54±2	37.93±7
Male 14/1	97.5±	869±	9.24	67.53	2.90	12.0	0.71058±13±	0.51214±3	17.98±1±	15.53±1±	37.95±3±
Main 14/3	267.2	1,183	—	—	—	—	0.70935±8	—	—	—	—
Main 14/6	267.3	1,263	—	—	—	—	0.71006±15	—	—	—	—
Main 15/0†	216.5	843	—	—	6.33	38.1	0.71090±4	—	17.87±2	15.63±1	38.08±3
Main 15/1	224.9	827	—	—	—	—	0.71253±8	—	—	—	—
Klip 3	155.5	1,843	—	—	2.71	19.5	0.70821±5	—	17.59±1	15.50±1	37.95±3

Concentrations in p.p.m. obtained by isotope dilution. Isotopic ratios are measured ratios uncorrected for age of emplacement. Errors on isotopic ratios are in-run 2σ standard errors of the means. Uncertainties from duplicates are 0.02, 0.004 and 0.15% for Sr, Nd and Pb isotopic ratios, respectively. Pb isotopic ratios are normalized to SRM981. $^{87}\text{Sr}/^{86}\text{Sr}$ for SRM981 = 0.71015 $\pm$ 4; $^{143}\text{Nd}/^{144}\text{Nd}$ for BCR-1 = 0.51270 $\pm$ 2. Sm and Nd in BCR-1 = 6.703 $\pm$ 0.013 and 28.934 $\pm$ 0.060 ppm, respectively.

* Aphanitic to microporphyritic hypabyssal kimberlite.

† Slightly altered, or contaminated samples.

‡ Average of duplicate concentration and/or isotopic ratio analyses.

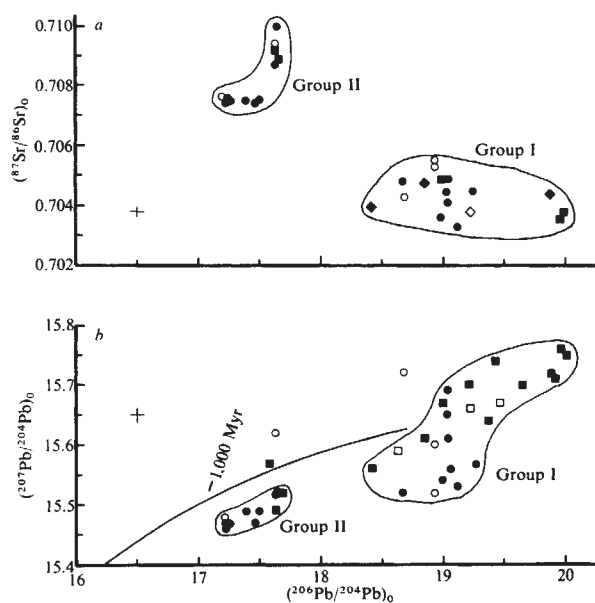


Fig. 1 Initial $^{207}\text{Pb}/^{204}\text{Pb}$ (b) and initial $^{87}\text{Sr}/^{86}\text{Sr}$ (a) plotted against initial $^{206}\text{Pb}/^{204}\text{Pb}$ for Group I and Group II kimberlites. Circles, this work; squares, previously published data^{2,3,7}; filled symbols, fresh samples; open symbols, altered or contaminated samples; diamonds in a, kimberlites for which Sr and Pb analyses were obtained on different samples. Error bars indicate approximate analytical uncertainty. Stacey-Kramers⁴² two stage growth curve plotted in b.

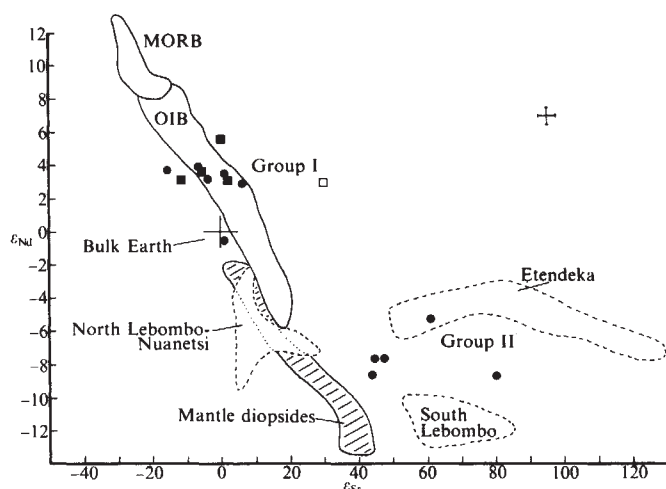


Fig. 2 $\epsilon_{\text{Nd}}/\epsilon_{\text{Sr}}$ for Group I and Group II kimberlites. Symbols for data points as in Fig. 1. Mantle array defined by mid-ocean ridge basalts (MORB) and oceanic island basalts (OIB)^{26,42}, and diopsides from peridotite xenoliths²⁴. Dashed fields enclose approximate ranges of Karoo basalts derived from enriched sources for three different regions of southern Africa²². Error bars indicate analytical uncertainty except for the Swartruggens sample. The large variability in initial $^{87}\text{Sr}/^{86}\text{Sr}$ in that kimberlite is similar to results obtained by Mitchell and Crocket⁴³, and could result from extreme sample heterogeneity or alteration.

$$\epsilon_{\text{Nd}} = \left[\frac{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{init. of sample}}}{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{t of CHUR}}} - 1 \right] \times 10,000$$

where t is age of kimberlite, $(^{147}\text{Sm}/^{144}\text{Nd})_{\text{CHUR}} = 0.1936$, $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}$ (present day) = 0.51264, and kimberlite analyses normalized to $^{143}\text{Nd}/^{144}\text{Nd}$ in BCR-1 = 0.51266, the Leeds value⁴⁴ applicable to previous work³. ϵ_{Sr} is analogous, with $(^{87}\text{Rb}/^{86}\text{Sr})_{\text{UR}} = 0.0827$ and $(^{87}\text{Sr}/^{86}\text{Sr})_{\text{UR}}$ (present day) = 0.7045.

lower initial $^{143}\text{Nd}/^{144}\text{Nd} = 0.51251$ (Table 1, Fig. 2). Group II kimberlites, however, have considerably lower initial $^{143}\text{Nd}/^{144}\text{Nd}$ isotopic ratios ranging from 0.51208 to 0.51228 (Table 1, Fig. 2) indicative of enriched source rocks, and tend to have lower Sm/Nd ratios (Table 1). These results are similar to those reported by DePaolo¹⁷ for a sample of the Kao kimberlite pipe, Lesotho, which Basu and Tatsumoto⁵ considered to reflect alteration. It is suggested here that the Kao analyses are likely to pertain to a different sample locality in light of the isotopic, petrographic, and age distinctions between Group I and Group II kimberlites. The Pb isotopic composition of Kao samples⁷, the isotopic compositions of other northern Lesotho kimberlites^{2,3,7}, the 90 Myr emplacement age of Kao¹¹, and the petrographic character of that kimberlite¹⁸ are indicative of a Group I affinity.

Small degrees of alteration and/or contamination by crustal xenoliths are virtually an unavoidable aspect of isotopic work on whole-rock kimberlites, but cannot be responsible for the distinctions between Group I and Group II kimberlites. Analyses of kimberlite samples that from petrographic evidence are altered and/or contaminated² do not generally show such major isotopic changes (Figs 1 and 2), although $^{87}\text{Sr}/^{86}\text{Sr}$ ratios can be considerably increased by interaction with groundwater³ (Fig. 2). The samples analysed for this work are as unaltered and uncontaminated as possible although Group I kimberlites are characterized by variable degrees of serpentinization and/or minor carbonatization. Group II kimberlites (with the exception of Swartruggens) tend to be fresher, are not serpentinized, and contain fewer xenoliths. Three samples of aphanitic to microphyritic hypabyssal kimberlite from Frank Smith, Jagersfontein, and Finsch (Table 1) are exceptionally fresh and devoid of xenoliths, and are most unlikely to be altered or contaminated. Relatively rapid emplacement of kimberlite would not favour large degrees of contamination by assimilation, and xenolith entrainment can be visually assessed. The high incompatible element contents of kimberlite (except for U and Pb) in conjunction with simple mixing calculations argue against contamination. Finally, it would indeed be coincidental if the observed coherent relationships between isotopic compositions, petrographic character, and emplacement ages for kimberlites in such a large geographical area could be explained by such processes.

The difference in isotopic character between the two types of kimberlite must reflect substantial chemical differences in their mantle source rocks. Group I kimberlites are derived from undifferentiated to slightly depleted sources relative to bulk Earth (Sr and Nd systematics), but with high U/Pb ratios. The Group I Frank Smith kimberlite could be derived from a distinct undifferentiated source with respect to Sr and Nd as previously suggested for most kimberlites⁴⁻⁶. However, considering that Frank Smith is the same age as the nearby Group II Newlands kimberlite, its isotopic character could also be explained by mixing of Group I and Group II magmas in a common 'plumbing system' during emplacement. This cannot be fully tested until further data are available.

Group II kimberlites are derived from enriched sources with high Rb/Sr and Nd/Sm, but low U/Pb ratios. The Sm-Nd systematics indicate that the enrichment event(s) must have occurred before 500 Myr ago (and more likely more than 1,000 Myr ago), and cannot have been caused by relatively young metasomatic or igneous events of less than 300 Myr such as recorded in some peridotite xenoliths¹⁹⁻²¹. Ancient enrichment of portions of the southern African subcontinental mantle is also documented by Sr and Nd isotopic compositions of some Karoo basalts^{22,23} (Fig. 2) and mantle-derived peridotite nodules in kimberlites^{20,23}. A possible alternative to ancient enrichment of source rocks is derivation of Group II kimberlites from subducted lithosphere containing a crustal component of continental origin. Although involvement of subducted oceanic crust in kimberlite genesis may be possible^{25,26}, the small amounts of pelagic sediments incorporated into a subducting slab would be unlikely to yield the appropriate isotopic

compositions²⁷, even over protracted periods of time. It is questionable whether subduction of coherent blocks of continental crust to the required depths is a feasible process²⁸, especially in the context of southern African tectonics.

The inverse correlation of Pb isotopic compositions with Sr and Nd isotopic compositions in kimberlite has also been documented in oceanic basalts from the Walvis Ridge²⁹ and opposes the positive correlation characteristic of most MORB^{30,31}. Richardson *et al.*²⁹ suggest that enrichment of Rb and Nd over Sr and Sm could be controlled by both melts and fluids, but that increased or decreased U/Pb ratios could be controlled by melts or fluids respectively. Non-lithophile behaviour of U in highly reducing conditions is characteristic of at least some enstatite chondrites³² and perhaps could be a feature of upper mantle enrichment events if CO₂/H₂O were sufficiently high. Alternatively, the Pb-isotopic systematics of Group II kimberlites could be controlled by high Pb/U minerals in the source rocks. K-richterite is a potential candidate for this role as it is likely to be more abundant than sulphide minerals in mantle rocks, and is characterized by low U/Pb and Sm/Nd and high Rb/Sr ratios (J. D. Kramers, personal communication). However, it may not be a stable phase at the depths of kimberlite genesis³³. Phlogopite is another obvious candidate although the few published Sm-Nd analyses indicate inappropriately high Sm/Nd (ref. 5), and little is known about its Pb isotopic character. Enrichment at the depths of kimberlite genesis may be reflected by different mineral assemblages than observed in metasomatized peridotite xenoliths. Though the mechanisms are unclear, low U/Pb coupled with high Rb/Sr and Nd/Sm might be characteristic of enriched mantle such as represented by the sources of Group II kimberlites and some oceanic²⁹ and continental basalts^{22,23}.

The recognition of ancient enriched upper mantle rocks apparently does not accord with isotopically defined two-reservoir mantle models³⁴⁻³⁶. At least three reservoirs are required, in addition to continental crust, as has been noted elsewhere³⁷⁻⁴⁰. One reservoir (depleted) is the source of most MORB. A second is apparently represented by the less depleted source regions of Group I kimberlites and oceanic island basalts. Zindler *et al.*⁴⁰ imply that volumetrically insignificant metasomatic components such as represented by sources of Group II kimberlites cannot be considered as a major long-term chemical reservoir; if so a third component has not been identified. In this regard both Group I and Group II kimberlites plot as clouds centred on the 'mantle plane'⁴⁰ but do not define a close fit. It is, therefore, arguable whether or not enriched subcontinental or suboceanic mantle could represent an additional reservoir. Resolution of this problem will probably require integrated Sr, Nd and Pb isotopic analyses of more mantle derived rocks.

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Evidence from early Proterozoic anhydrite for sulphur isotopic partitioning in Precambrian oceans

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Relative to meteoritic sulphur, sulphur in the contemporary exogenic cycle is marked by a depletion of ³⁴S in biogenic sulphide that is balanced by excess ³⁴S in sulphate of the oceans and evaporites^{1,2}. The onset of this isotopic partitioning during the Precambrian required sulphate in the ocean in sufficient concentration to permit significant isotopic fractionation during biogenic reduction^{3,4}; and the evolution of sulphate-reducing bacteria⁵⁻⁷. The conclusion that partitioning existed at 2,300 Myr has been derived from strongly ³⁴S-depleted sedimentary sulphide of this age from South Africa⁴. I report here on ³⁴S-enriched, stratiform, nodular anhydrite of similar age from Canada, which supports partitioning at this time. Strontium isotope data on these samples indicate that the anhydrite was derived from continental brines, possibly in the inland portion of a continental sabkha. This requires that the reported enrichment in ³⁴S be considered a minimum for seawater sulphate at 2,300 Myr.

Since sulphate is isotopically homogeneous in the ocean⁸ and is precipitated as CaSO₄ with relatively minor fractionation⁹⁻¹¹, sulphate evaporites are the medium of choice for identifying and measuring isotopic partitioning in ancient oceans. The oldest stratiform anhydrites reported to date have maximum ages of ≈1,300 Myr and are enriched in ³⁴S with a mean δ³⁴S of +17.5‰ (ref. 12). Stratiform barites >3,200 Myr old from several localities have δ³⁴S values close to meteoritic sulphur at 0‰ (ref. 13). Thus, isotopic partitioning commenced at some time between these far-spread dates, but the rarity of stratiform sulphate in the Precambrian has prevented better definition.

The alternative approach for identifying partitioning is to search for the oldest biogenic sulphide, that is, depleted in ³⁴S (refs 14-19). Sulphide that is consistently and strongly depleted in ³⁴S, to -30.6‰, has been found⁴ in basal carbonaceous shales of the ~2,300 Myr Timeball Hill Formation, part of the Transvaal Supergroup of South Africa (Fig. 1). This gives a minimum age for the onset of partitioning, provided that the data are representative of the exogenic cycle of the period. The presence

of stratiform, nodular, anhydrite of similar age in the Huronian of Ontario provides a means for supporting or denying partitioning at 2,300 Myr.

Within the constraints of available geochronological data, the Huronian and Transvaal Supergroups are of similar age. Volcanic rocks underlying the Transvaal are dated by the U-Pb method at $2,460 \pm 120$ Myr (ref. 20). Rb-Sr ages for the Ongeluk lavas are $2,224 \pm 21$ Myr (D. Crampton in ref. 21) and for the Timeball Hill Shales $2,263 \pm 85$ Myr (ref. 22). The Huronian has been considered to be $\sim 2,300$ Myr on the basis of underlying 2,500-Myr granites; a 2,166 Myr age for diabase intruding the Huronian; and a Rb-Sr date of $2,288 \pm 87$ Myr for Gowganda sedimentary rocks²³. A recent U-Pb date on granite intruding the lowermost Huronian strata indicates a minimum age for the latter of $2,333 \pm 32$ Myr (ref. 24).

The Huronian is dominated by coarse clastic sediment, much of which is from felsic plutonic terrane to the north of the present outcrop²⁵. This was carried south and, in part, deposited in river systems and the remainder in the sea. The nodular chert, anhydrite and gypsum, described by Wood^{26,27}, is from the lower part of the Gordon Lake Formation in the Flack Lake area. This unit is composed of reddish, grey and green siltstone, with fine-grained quartzite. Some of this material may be of loessic origin²⁷. There are red, green and purple chert beds and laminae and rare beds of limey siltstone. Because of the presence of graded bedding, haematite oolites, chert, intraformational breccia and sedimentary intrusions, Wood believed that the unit is a tidal flat deposit analogous to the present Colorado River delta. He suggested, by analogy to more modern sediments, that the nodules formed in a lagoonal or supratidal environment during early diagenesis. Frarey²⁸ also found tidal or supratidal features in the Gordon Lake indicating

Table 1 Analytical data for Gordon Lake Formation anhydrite samples

Depth (feet)	$\delta^{34}\text{S}$ (‰)	$^{87}\text{Sr}/^{86}\text{Sr}$	Sr (p.p.m.)	Rb (p.p.m.)
1,776	+15.1	0.7365*	1,150	2.2
1,813	+15.6	0.7379	1,436	0.0
1,844	+12.4	0.7458*	1,482	1.9
1,851	+12.3	0.7459	1,309	0.0
1,867	+13.3	0.7419	1,506	0.2

* Corrected for ^{87}Sr formed from ^{87}Rb since deposition of anhydrite.

a nearshore marine, to littoral, possibly lagoonal, environment. There is evidence in the Huronian record of cycling from glacial to tropical climates²⁹. The presence of aluminous minerals in the Lorrain Formation, below the Gordon Lake, has been taken to indicate tropical weathering³⁰.

The nodules are not well preserved in outcrop. The analysed anhydrites (Table 1), which contain no gypsum, come from the Canadian Johns Manville drill hole 157-68-1 near Flack Lake, Ontario. Twenty intersections of anhydrite as much as 3 inches thick occur between 1,769 and 1,904 feet. The petrography of the nodules and host rocks have been described by Wood²⁷. Thin section examination of the samples (F. W. Chandler, personal communication) shows irregular, inclusion-free anhydrite nodules in laminated mudstone. There is minor displacement of mudstone laminae around the nodules. These features are consistent with growth by displacement of soft, fine-grained sediment, which is the most commonly considered mode of origin for nodular anhydrite of Recent and Phanerozoic age³¹.

Sulphur isotope analysis was done by the method of Thode *et al.*³² and $^{87}\text{Sr}/^{86}\text{Sr}$ as described by Wanless and Loveridge³³. Sr and Rb was analysed by isotope dilution. The strontium isotope measurements were made to establish whether or not the anhydrite is of marine origin. Seawater at 2,300 Myr is estimated to have $^{87}\text{Sr}/^{86}\text{Sr} \approx 0.703$ (ref. 34); a significantly higher value should indicate continental waters.

Patterson and Kinsman^{35,36} present a model for a modern, coastal sabkha wherein anhydrite that is deposited immediately inland from the shore is precipitated from groundwater of marine origin. Further inland the anhydrite comes from groundwater of continental origin (Fig. 2). The strontium isotope data for the Gordon Lake anhydrites at ~ 0.74 are more consistent with formation in the inland portion of a coastal sabkha from continental groundwaters than from seawater. That tidal features have been identified in these sediments is not in conflict with this inference. Advance and regression of the sea across a relatively flat sabkha surface can cause interbedding of continental and marine sediment facies.

The $^{87}\text{Sr}/^{86}\text{Sr}$ values of ~ 0.74 for the Gordon Lake anhydrites indicate derivation from a ^{87}Rb -, ^{87}Sr -rich source. The Archaean K- and Rb-rich granites that, in part, underlie the Huronian²⁸ would have been several hundred million years old at the time of deposition of the Gordon Lake Formation; sufficient for generation of significant amounts of ^{87}Sr . The strontium isotopic ratios of the anhydrites are comparable with the maximum value of 0.7385 for Canadian river waters³⁷. It is presumed that some strontium and sulphate from seawater was precipitated in rain over this Huronian sabkha and hinterland. However, it is improbable that groundwater with an isotopic ratio as high as ~ 0.74 was derived by simple mixing of seawater strontium at ~ 0.70 with continental groundwater having a ratio $\gg 0.74$. More likely there was ion exchange of ^{86}Sr for ^{87}Sr during weathering of biotite³⁸, resulting in the preferential loss of ^{87}Sr from this mineral³⁹⁻⁴¹. Redbeds are found in the Gordon Lake Formation⁴². The iron for redbeds formed in sabkha and desert environments may come from the intrastratal solution of iron-rich silicates, such as biotite⁴³; a process that would facilitate the release of ^{87}Sr . Unfortunately, the lack of strontium isotope data from younger sabkha anhydrites does not allow consideration of alternative models for

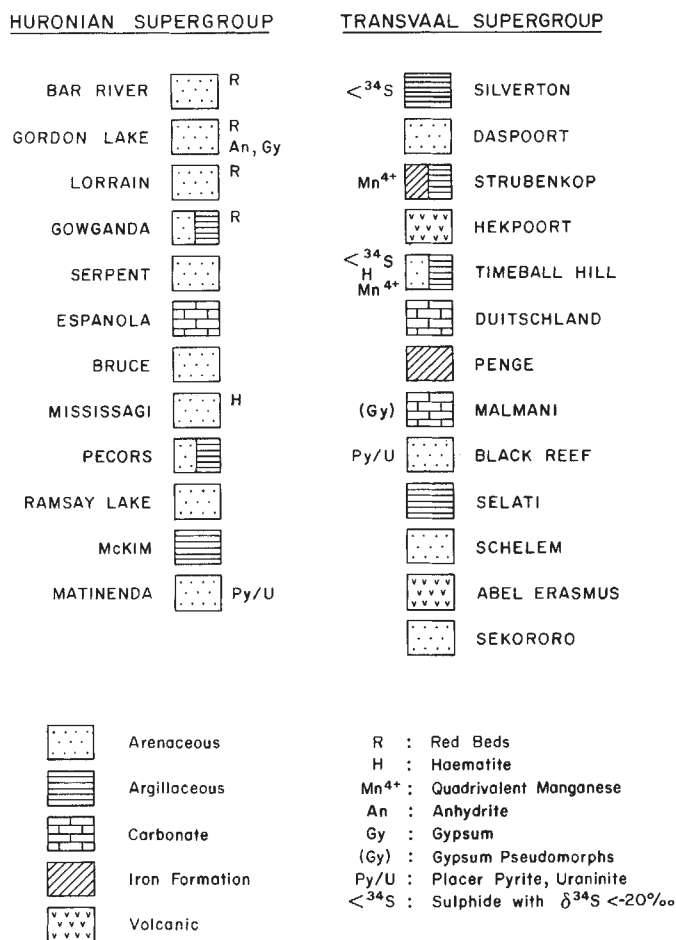


Fig. 1 Features of units comprising the Huronian and Transvaal Supergroups. In part based on information from refs 4, 42, 46, 47, 58.

the high ratio values, such as post-depositional isotopic equilibration with ^{87}Sr -rich formation waters.

The possibility that these anhydrites were derived from continental brines places constraints on the interpretation of the sulphur isotope data. Sulphate from sea spray is a major constituent of atmospheric aerosols over maritime regions⁴⁴ and is precipitated over adjoining land areas. However, in addition to seawater sulphate, groundwater contains sulphate from other sources, such as the oxidation of sulphides or volcanic SO_2 . These additions will reduce the $\delta^{34}\text{S}$ values of groundwater⁴⁵ by an unknown amount. In the modern Abu Dhabi sabkha this is quite minor with the anhydrite derived from continental brines averaging 1‰ less than ocean water¹¹. The isotopic data shown in Table 1 and Fig. 3 indicate that those anhydrite samples with most ^{87}Sr , which may represent marginally more 'continental' groundwaters, are less enriched in ^{34}S . Thus the high $^{87}\text{Sr}/^{86}\text{Sr}$ values for the anhydrites require that the $\delta^{34}\text{S}$ values, in the range +12.3 to +15.6‰, be considered as minimum values for the ocean of that time.

The Huronian and Transvaal Supergroups are similar in that their lower strata contain pyritic-uraniferous placers (Fig. 1), whereas their upper strata have haematite, manganese oxides and, for the Huronian, redbeds. These and related features have been taken as evidence for a fundamental change from an oxygen-poor to an oxygenated atmosphere/hydrosphere during deposition of the units^{28,46,47}. Both the ^{34}S -enriched Huronian anhydrite and the strongly ^{34}S -depleted Transvaal sulphide are from the upper parts of these units that show evidence of contemporaneous oxidation. This is consistent with the proposal that oxygenation of the atmosphere above trace levels and development of the oceanic sulphate reservoir were synchronous events⁴. However, these data are not proof of this proposal and further testing of 3,000–2,200 Myr stratiform sulphate and sulphide is required.

From the time that there existed both an oceanic sulphate reservoir and sulphate-reducing bacteria, there should be widespread evidence in sedimentary rocks for sulphur isotope partitioning. The present data add to the evidence previously compiled⁴ for partitioning from ~2,350 Myr onwards. However, what evidence should there be if the reservoir developed

before these bacteria, or vice versa? The first case has been discounted⁴ because of the lack of consistent evidence in the Archaean for inorganic reduction of sulphate. Considering the second case, sulphate-reducing bacteria developing before an oceanic reservoir, there may be examples of isotopic partitioning within restricted sulphate-rich basins or seas, since sulphate was present in significant concentration in some bodies of water during the early Archaean^{13,48–56} and later Archaean⁵⁷.

I thank Mr G. Bennett for facilitating the sampling of drill core, Dr C. E. Rees for sulphur isotope analyses, Dr O. van Breemen and Mr W. D. Loveridge for strontium isotope dilution analyses, and Dr F. W. Chandler for petrographic examination. Drs K. D. Card, F. W. Chandler, I. R. Jonasson, D. F. Sangster, R. I. Thorpe and O. van Breemen provided critical comments on the manuscript.

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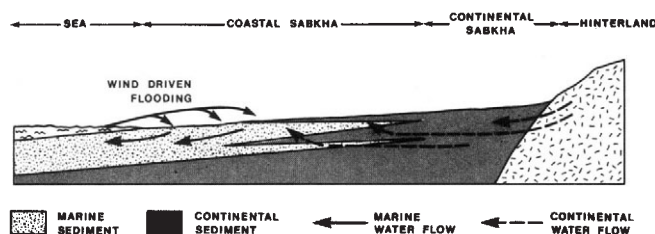


Fig. 2 Hydrogeological model of a coastal sabkha. After refs 35, 36.

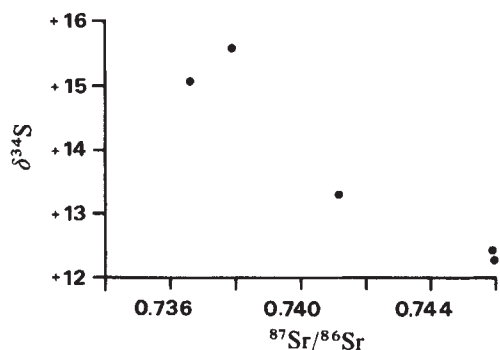


Fig. 3 $^{87}\text{Sr}/^{86}\text{Sr}$ versus $\delta^{34}\text{S}$ for Gordon Lake Formation anhydrites.

Radiographic observation of chamber formation in *Nautilus pompilius*

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Radiographic observation of four immature *Nautilus pompilius* shows that new chamber formation commences when approximately half of the cameral liquid of the last formed chamber has been osmotically removed. At this time, the septal mantle is rapidly moved forward in the body chamber, where it re-attaches to the internal shell wall and begins calcification of a new septum. Throughout this time, apertural shell growth rates remain constant. The time between successive chamber initiations increased during ontogeny; in the four specimens monitored here, time between two successive chamber formation events ranged between 85 and 132 days and showed no apparent lunar correlation.

Externally-shelled cephalopods, such as *Nautilus*, produce neutral buoyancy with the use of air and liquid-filled chambered shells. As the animal grows, it must periodically enlarge the chambered shell portions if neutral buoyancy is to be maintained. Denton and Gilpin-Brown¹ outlined the major steps involved, and the terminology used here is adapted directly from their study. New chamber formation initiates with forward movement of the body to produce a liquid-filled cavity at the rear of the body chamber. This space is then sealed off with a calcareous septum, and the enclosed liquid removed osmotically by a central strand of tissue, the siphuncle, which runs through each chamber. We have further studied this process of new chamber formation in one species of *Nautilus*, *N. pompilius*, by periodically radiographing immature specimens maintained in surface aquaria. Our results give new information on the relationship of body movement, apertural growth, and chamber liquid levels during new chamber formation.

Four *N. pompilius* were captured in the Philippine Islands and airshipped to the New York Aquarium, where they were kept in a recirculating seawater system maintained at 18–20 °C. The animals were subjected to alternating 12-h periods of faint light and complete darkness, fed daily, and weighed and radiographed weekly or bimonthly. It has previously been shown that frequent or long-term radiography has no apparent detrimental effect on aquarium-maintained *Nautilus*². The *Nautilus* were weighed on a top-loading balance, after first being drained of liquid by shaking the shell with body chamber pointing downwards, until no further seawater drained out. Even using this technique, repeated weighings of the same specimens showed airweights to be accurate only to within ± 2 g.

The weight records and corresponding radiographic observations (listing stage of chamber development for any given observation) are shown in Fig. 1. Ten chamber initiations were observed among the four specimens during the 10-month observational period. The shortest period between successive chamber initiations was 85 ± 3 days; the longest, 132 ± 7 days. Intervals between chamber formation events increased in larger volume chambers, as had previously been predicted^{3–5} on the basis of change in siphuncular surface area with growth. In each observed case, new septal formation occurred when the level of liquid in the latest chamber descended to a point where it was no longer in direct contact with the siphuncle. This occurs

when a chamber is slightly more than half emptied (Fig. 2). A similar correlation between this liquid level (transition from coupled to decoupled cameral liquid emptying), and the onset of new chamber formation also occurs in *N. macromphalus*².

New septa are produced by the posterior or septal mantle. Dissection of living *Nautilus* shows that during septal calcification, the septal mantle is directly in contact with the septal surface. Through the use of radiography the relationship between calcification and chamber emptying can be directly observed.

A new chamber will not be emptied of liquid until the septum is sufficiently thick to withstand the great pressure difference between ambient pressure, and the virtual vacuum in the chamber that occurs when the first cameral liquid is osmotically removed¹. In wild-caught *Nautilus macromphalus* emptying commences when the calcifying septa reach ~60% of their eventual thickness²; in our specimens, emptying began when the calcifying septa reached half of their ultimate thicknesses (Fig. 3). Completion of septal calcification therefore does not occur before cameral liquid emptying, but instead takes place weeks after the initiation of emptying. The completion of septal calcification appears to coincide closely with a liquid volume in the enclosed chamber dropping below a level which allows direct contact with the siphuncle. At this time, (the coupled-decoupled transition of Denton and Gilpin-Brown), the posterior mantle moves away from the now-completed septum. The timing of this movement can be estimated from a series

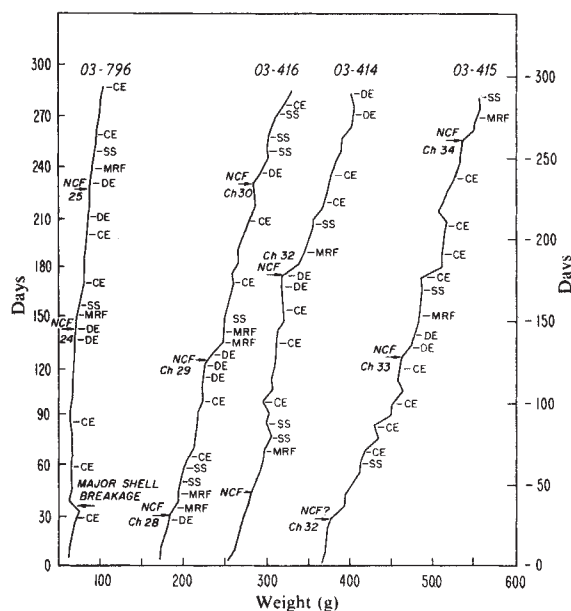


Fig. 1 Weight changes (air weight) of four aquarium-maintained *Nautilus pompilius*. Symbols represent selected radiographic observations during the study. CE, Coupled chamber liquid emptying, which occurs when cameral liquid in the last formed chamber is in direct contact with the connecting ring of the siphuncle; during CE the last-formed septum is still being thickened (calcified). DE, Decoupled emptying, which occurs when liquid level drops below the connecting ring; in this case, which occurs when the chamber is more than half emptied of liquid, the remaining cameral liquid is carried onto the siphuncle because of the wettable properties of the chamber walls. The transition from CE to DE coincides with initiation of a new chamber by forward movement in the body chamber of the septal mantle. MR, Formation of the mural ridge in radiographs indicates that the septal mantle has moved forward in the body chamber from its previous position against the face of the last-formed septum. SS, Pre-emptying septal secretion, where the new septum is being formed by the secretion of nacreous calcium carbonate. In this stage, the septum is still too thin (and weak) to allow emptying. When the septal wall is sufficiently thick to withstand the great pressure differential between ambient pressure (high) and chamber pressure (low), coupled emptying will begin.

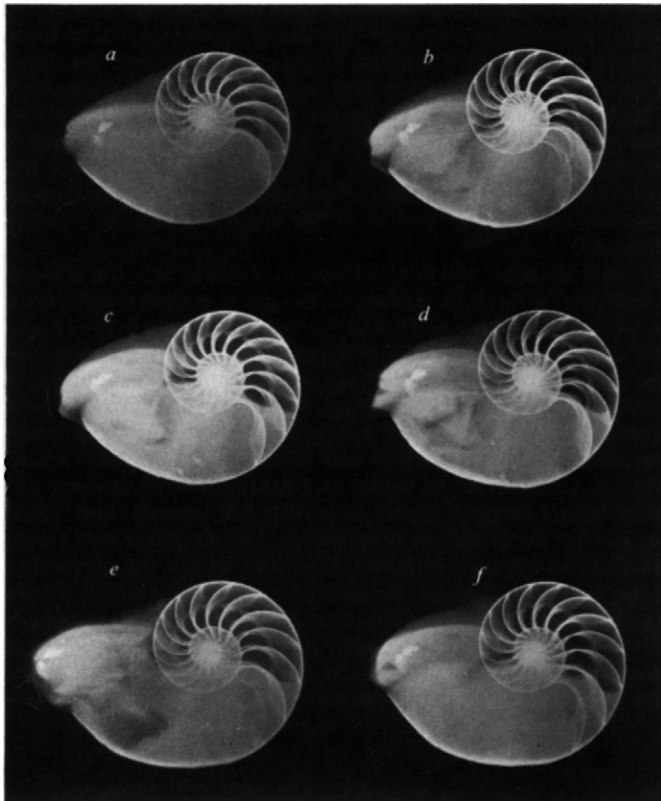


Fig. 2 Growth and chamber formation in specimen 416, showing two successive chamber formation events. Dates of radiographs: *a*, 7 December 1981, illustrating DE stage; at the time of this radiograph, septal mantle is moving forward in the body chamber towards its new attachment site. *b*, 31 December 1981; in the 3 weeks since the last photo, the septal mantle has moved forward, formed the mural ridge, and is in this radiograph shown calcifying a new septum (SS stage). *c*, 20 January 1982; coupled emptying. *d*, Transition from CE to DE, taken on 11 March 1982; note that the liquid in the preceding chamber, which in the first radiograph occupies about half the chamber volume, is completely gone. *e*, 8 April 1982; formation of a new septum (SS stage). *f*, 23 April 1982; coupled emptying of the last formed chamber.

of radiographs, since it must take place between the time of final septal calcification, and the time of first appearance of a new septum anterior to the newly completed septum. Based on the radiographic observations, it appears actual movement takes, at most, between 10 and 20 days depending on size. Actual movement could be much faster than this, however, since radiography only can bracket the timing between two calcification events; the mantle itself is invisible in the radiographs. Forward movement is thus a 'rapid' process, lasting ~10% or less of the entire chamber formation period.

Martin *et al.*⁶ proposed that new chamber formation is detectable by periodic weight increases, the rationale being that forward movement of the septal mantle creates an enlarging fluid filled space between the advancing body and the last-formed calcareous septum; the secretion of new cameral liquid into the enlarging space would then show up in careful weight records. If, however, the new cameral liquid was excreted from the body, and not immediately replaced by uptake of fluid from the sea, no change would be observed. Our observations suggest that new chamber formation may be characterized by a weight signature. In four chamber formation events (Fig. 4), the first evidence of new chamber formation (as observed in radiographs), was preceded by rapid weight gains of between 3 and 9% of the total air weight. These periods of increasing weight lasted between 10 and 20 days, and were themselves usually preceded by a period of less-than-normal, or even declining weight gain. Neither the period of low weight gain, nor the period of rapid weight gain immediately preceding the

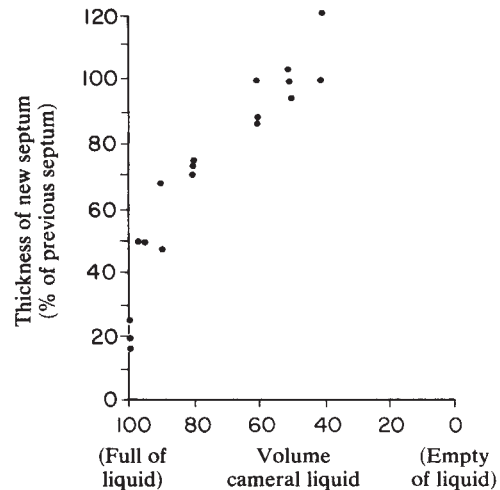


Fig. 3 Chamber volume (liquid content, with 100 indicating that the chamber is filled with liquid, and that no emptying has yet taken place) plotted against thickness of the last formed septum, which is measured as a percentage of the thickness of the preceding septum. Since each new septum is ultimately slightly thicker than the preceding one, the value can be >100%. This graph indicates that emptying of a chamber begins when the septum is about half calcified (about half of its ultimate thickness), and the final thickness is achieved when about half of the liquid has been emptied from the chamber. This point coincides with the transition from CE to DE, presaging forward movement of the mantle to another new septal site.

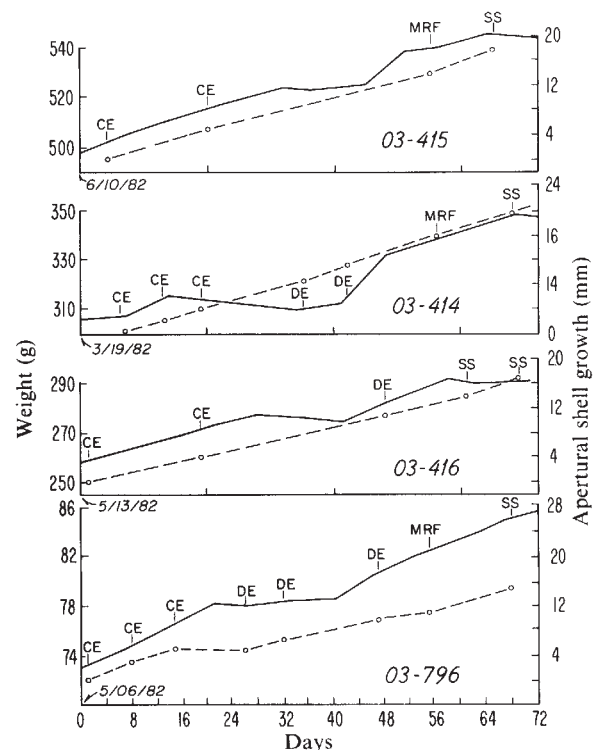


Fig. 4 Weight change (solid line, with weights on left vertical axes) and apertural shell growth in mm (dashed line, with distances from an arbitrary starting point on right vertical axes) plotted against time for four chamber formation events in four different *Nautilus*.

calcification of a new septum seemed to be related to changes in rate of apertural shell growth, since apertural shell rate remained constant during this time (Fig. 4). These rapid weight gains may be due to the creation of a new liquid filled space behind the advancing septal mantle. This liquid is chemically different from seawater and is secreted either by the septal

mantle itself, or by the new strand of siphuncular cord^{1,7}. Because of the imprecision of air-weighing, however, such weight changes would only be detectable in *Nautilus* producing chambers of ~10 cm³ volume or more, and only in conditions of known and constant weight change due to feeding. Even in controlled aquarium conditions, attaining the latter is difficult, and as seen from Fig. 1, many weight changes of similar magnitude occurred between septal formation events, while some actual formation events did not show this trend. Identifying chamber formation events solely on the basis of weight change patterns, without the benefit of radiography, would be highly inaccurate. Nevertheless, just such evidence has been used to derive rates of chamber formation in *Nautilus*⁸.

The chamber formation cycle in *Nautilus* can thus be viewed as a highly organized and regular process. The shell aperture and the soft parts are constantly growing forward; the septal mantle, however, remains fixed against the various septa for over 90% of the animal's life. It only moves forward when the last formed septum is completely calcified; full calcification coincides with the chamber being half emptied of liquid. The septal mantle is then pulled forward by muscular action, leaving behind a liquid filled space that will become the cameral liquid

of the next chamber. It reattaches and begins secreting a new calcareous septum, that in turn will be abandoned when completely thickened. The osmotically-induced removal of liquid from the chambers occurs at a rate that balances density increases of growing shell and tissue.

Although the period between chamber formation events could be as small as 30 days or less for the low-volume, early chambers produced by very young *Nautilus*, our observations suggest that chamber formation takes very much longer than a month during most of an animal's ontogenetic development. The four *Nautilus* maintained in this study were raised in conditions very different from their native habitat, perhaps resulting in growth rates very different from those in nature. The apertural growth rates ranged between 0.15 and 0.25 mm day⁻¹, values which are comparable with growth rates of similarly sized *N. macromphalus* grown in aquaria^{2,6}.

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Lucy's lower limbs: long enough for Lucy to be fully bipedal?

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The recent attempt to show that the Hadar australopithecine female 'Lucy' (AL 288-1) had hindlimbs too short to allow a modern pattern of striding bipedal gait¹ has important implications for understanding the origin of bipedalism, if not for the more general problem of hominid origins. Combined with previous claims that Lucy had a forelimb unusually long in proportion² and ape-like in morphology³, the additional contention of a relatively short hindlimb would suggest a very different pattern of gait from the norm of today because the effectiveness of the pendulum action of the lower limb during stride is a function of the amount of mass in the limb⁴, and because a short hindlimb would necessitate a short stride length¹. Yet, these contentions seem contradicted by the analyses of Lucy's pelvis (and the innominates of other australopithecines)^{4,5} that indicate a similar pattern of muscle use and imply a lack of significant gait differences. Are Lucy's legs too short to allow an effective stride, or is there a different solution to this contradiction? I propose here that there is.

Jungers¹ attempts to answer the question of whether the high humeral/femoral index of AL 288-1 reflects a relatively long forelimb or a relatively short hindlimb, by expressing each limb length as a deviation from the allometric relation of the limb length to body weight in the African apes. These deviations are compared with deviations for skeletal measures of a pygmy chimpanzee of known weight, an Mbuti Pygmy (MMC-18) of estimated weight and a European sample of known weight. The results show a femoral deviation for Lucy like that of the chimpanzee and much less than that of the human, but a humeral deviation like the human's and in the opposite direction to the chimpanzee, leading to the conclusion that the evidence does not support an interpretation of "a fully modern adaptation to bipedalism". I contend that this conclusion comes from a misinterpretation of the data, and that the hindlimb of

AL 288-1 is the length one would expect in a hominid of her size.

Part of the problem comes from the Mbuti Pygmy weight used, as it is actually no more than a guess⁶; the maximum estimate of 30.9 kg is the average weight (W_t) for a 12-yr old Pygmy female⁷. To estimate her weight more accurately, a best fitting power curve regression for the $W_t = f(H_t)$ relation in 81 female Efé Pygmies was determined ($r = 0.71$). (Data were collected by the Harvard Ituri Project and were made available by R. C. Bailey, N. R. Peacock and members of the project.):

$$W_t = 0.00011 \times H_t^{2.592}$$

In this and other equations, linear measures are in centimetres and weight is in kilogrammes. Height was determined for this female using the tibia and femur⁸ that are published in the description of her skeleton⁹ and her weight was calculated to be 41.4 kg. The corresponding deviation of her femur length from the ape allometric curve, 14%, is considerably less than Jungers thought.

Interestingly, the Mbuti Pygmy femoral deviation is only one-third the deviation for European females¹, raising the possibility that this deviation decreases with decreasing body size. As AL 288-1 is smaller than any living Pygmy by virtually every measure of the trunk or lower limb, the expected femoral deviation from ape allometry for humans this small is of some interest, if only to determine whether the femur of AL 288-1 is smaller than expected for a human of Lucy's diminutive weight. For this purpose, heights were ascertained from femoral and tibial measurements⁸ for 15 published Pygmy skeletons^{9–14} (6 females, 9 males). Weights were calculated for these specimens using the female power curve relation given above, and from a similar power curve calculated for 93 Efé males (the Harvard Ituri Project data) ($r = 0.67$):

$$W_t = 0.00062 \times H_t^{2.241}$$

Using these weights, femoral and humeral deviations from the African ape allometric curve were calculated for the sample. Each set of deviations was used in a regression of deviation as a function of weight. For the humeral deviations the correlation ($r = 0.56$) and regression coefficient were not significant. However, the femoral deviations gave a significant correlation ($r = 0.83$) and regression coefficient

$$D = 0.00042 \times W_t^{2.828}$$

Using this formula, and accepting the 27.3 kg estimate published for Lucy² as Jungers does (this weight is also almost exactly what the male Pygmy formula predicts), the expected femoral deviation for a human of Lucy's weight is 4.9%. The actual AL 288-1 femoral deviation calculated by Jungers is between -2.1% and 0.1%. Thus, Lucy's femur is within about 5% the deviation one would expect in a human of the same size. Instead of using the Pygmy sample alone, an alternative calculation of the expected deviation was attempted using the mean male and female European figures from Jungers¹ and the male and female Pygmy means from the data discussed here. The best fitting power curve for these four points gives an expected femoral deviation of 6.5% for a human of Lucy's size, confirming the above.

Because weight is an estimated quantity for all of the critical data of this (and Jungers') analysis (the Pygmies and the australopithecine in question), a means of ascertaining relative limb length independent of body weight, and independent of the limbs themselves, would be useful. Limb length is traditionally expressed as a percentage of trunk length¹⁵, and some of the vertebrae are preserved for both Lucy and a second female specimen from Sterkfontein, STS 14. The vertebrae comprise the trunk; their heights (and more so the summed heights of each vertebral segment) are significantly correlated with trunk length and body height¹⁶. For instance, the correlation of the full lumbar segment height with skeletal height for six Pygmies with published skeletal heights (that are not estimated from the formula applied to limb lengths)^{11,14} is 0.77 ($P = 0.01$), and the correlation of L3 height (the only lumbar measurable for AL 288-1⁵) with skeletal height is 0.68 ($P = 0.03$). For comparison, the corresponding femur length correlation for this sample is 0.76 ($P = 0.02$). It therefore seems appropriate to use these vertebral dimensions as estimates of trunk length in establishing an independent measure of relative limb length.

Table 1 reports the lengths of the humerus and femur directly, as a percentage of the ventral height of the L3, and as a percentage of the summed ventral heights of the five caudal-most lumbar. The two australopithecines with these data are compared with European Neanderthals (partly because they are said to be short-limbed), all of the published Pygmies with these data¹¹⁻¹⁴, and a sample of Michigan Amerinds. Relative

humerus length for Lucy is close to the Pygmy mean. For both Lucy and the Sterkfontein female, relative femur length is below both the modern means and the Neanderthal mean. However, the difference is not great; relative to the L3, both australopithecines are only 1.3 standard deviations below the Pygmy mean and 1.5 standard deviations below the Michigan Amerind mean, while relative to the total lumbar segment STS 14 (and by inference Lucy) is 1.4 standard deviations below the modern means. Perhaps more importantly, the australopithecines are within the observed range of the Pygmy sample.

Clearly, the femora of AL 288-1 and STS 14 are very short. However, relative to estimates of trunk length they are within the human range, and it would appear that the femora are close to the length expected for humans as small as these diminutive australopithecine females. The humerus of AL 288-1 does not appear to be particularly short, its relative length being close to the Pygmy mean.

While both of Lucy's limbs are within the human range, no individual combines Lucy's proportions. Whether one prefers to call her arms long relative to her legs or her legs short relative to her arms (a better description considering the limb lengths relative to the trunk), the fact is that Lucy had a good deal of mass in her forelimb, and because of the consequent muscularity probably also in the upper trunk. In considering the mechanism of lateral balance during stride, the moment of this mass about the acetabulum was large. This probably explains the very efficient development of the lateral balance mechanism in australopithecines⁴—an example of skeletal/muscular differences that reflect the same pattern of gait. The morphology of the pelvis and femur clearly indicate that this pattern was exclusively bipedalism, irrespective of relative limb length¹⁷. I further suggest, however, that the allometric consequences of this human gait pattern provide a prediction of relatively short hindlimbs in very small hominids with an effective use of stride during bipedal locomotion. For this reason, and because both australopithecines have relative femoral lengths within the modern human range, Lucy's limb proportions cannot be construed as evidence against a fully modern pattern of bipedalism. While I agree with Jungers that "dramatic hindlimb elongation . . . emerges as one of the major evolutionary changes from *A. afarensis* to modern humans", this appears to have been an

Table 1 Limb length in proportion to vertebral body heights

	Ventral body height		Humerus length	H/V	H/SV	Femur length	F/V	F/SV
	L3	Sum for inferior 5 lumbar						
Australopithecines								
AL 288-1	19.3		236.8	12.3	(2.43)*	280	14.5	
STS 14	19.7	97.4				285	14.5	2.93
Neanderthals								
La Chapelle	27.6		313	11.3		430	15.6	
La Ferrassie 1	28.8		340	11.8		458†	15.9	
Shanidar 1	25.0					462†	18.5	
Shanidar 3	26.6	134.7	314	11.8	2.33			
Pygmies								
$\bar{x}$	22.1	110.3	267.1	12.1	2.43	364.2	16.6	3.31
σ	1.6	6.8	16.0	1.1	0.18	22.9	1.6	0.27
Minimum	20.0	98.0	238	10.3	2.09	326	14.2	2.86
Maximum	25.0	121.0	283	14.0	2.75	391.5	19.4	3.79
Michigan Indians ($n = 14$)								
$\bar{x}$	27.2	136.2	324	11.0	2.35	446	16.4	3.26
σ	1.4	5.9	18.8	0.9	0.16	21.9	1.3	0.23
Minimum	24.0	123.9	285	10.6	2.14	400	14.9	3.01
Maximum	29.6	147.8	352	13.9	2.69	481	19.8	3.83

The ratios are humerus length compared with the ventral height of the lumbar body (H/V), and compared with the summed ventral heights of the inferior five lumbar bodies (H/SV), and the same two ratios for maximum femur length. All measurements are in millimetres.

* Uses STS 14 vertebral sum as an estimate for AL 288-1.

† Determined by E. Trinkaus.

allometric consequence of increasing body size expressed in a hominid with a pattern of gait widely divergent from that of the African apes.

On the other hand, because of the unique proportions of Lucy's limbs relative to each other, the biomechanical requirements of this gait pattern differed somewhat from those for living people. It is possible that Lucy's limb proportions correspond to a different, perhaps broader, locomotor repertoire than is the case today. If, as claimed above, this difference does not lie in the bipedal gait pattern evidenced by the pelvic and hindlimb adaptations, contra Jungers it might be productive to focus on the unique aspects of the australopithecine forelimb.

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Rapid motion aftereffect seen within uniform flickering test fields

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Prolonged viewing of a moving pattern selectively elevates the threshold for a pattern moving in the same direction¹ and induces the classical motion aftereffect (MAE). The aftereffect is seen as a slow drift² in the opposite direction, which is visible even with the eyes shut^{2,3} or while viewing a uniform field^{3,4}. However, as we report here, a strikingly different aftereffect is seen when the test field is uniform and sinusoidally flickered: the field is filled with rapid motion in the direction opposite the adapting motion. This flicker MAE has distinct properties: the adapting grating must be of low spatial frequency; the effect is promoted by high contrast and high temporal frequencies of both adapting and test stimuli; and the aftereffect does not transfer interocularly. In all these respects the flicker MAE differs from the traditional MAE. Motion detectors have been identified in human vision by the threshold detectability^{5,6} and discriminability⁶ of moving patterns and by selective adaptation. The flicker MAE selectively taps a class of transient motion mechanisms that are selective for rapid motion and low spatial frequency. Uniform flicker is an effective stimulus for these mechanisms. It thus appears that the human visual system contains at least two distinct classes of mechanisms for sensing motion.

Stimuli were displayed on a cathode ray tube (CRT) that had a 6 deg diameter field of constant mean luminance (45 cd m⁻²) with dark surround. The observer stared at a central fixation point and saw a vertical sinusoidal grating that moved rightwards at a rate expressed in Hz. Periodically the adapting grating was replaced by spatially uniform sinusoidal flicker, whose rate (in Hz) was typically the same as that of the adapting grating. The observer pressed a button the instant that the rapid leftward motion aftereffect disappeared from the flickering field. This duration provided a measure of aftereffect strength. Most measurements were done with four to six observers; the results shown here for one or two observers are typical.

For a better impression of the aftereffect, consider the following. The observer adapted to a high-contrast, vertical grating of 0.4 cycle per deg moving rightwards at 8 Hz. The grating filled a 25 deg diameter field, of constant mean luminance. When the adapting grating was turned off, spatially uniform test flicker was presented in only the central 5° of the field. The observer reported that the rapid motion aftereffect was seen only in this central area. However, the aftereffect was seen over the entire field when the flickering region was increased to 25 deg diameter. Thus, the rapid motion aftereffect is seen only on flickering regions, and a very large flickering field may elicit the aftereffect.

Measurements with the 6 deg field demonstrate that the aftereffect is produced only with low spatial frequencies. Figure 1a shows that the aftereffect decreases as the spatial frequency of the adapting grating increases to 3.0 cycles per deg. When the adapting pattern was raised to the next step (4.2 cycles per deg), observer M.C. saw no aftereffect and M.G. saw a fleeting effect that was too brief to measure. The adapting and test rates were 8 Hz, and each adapting grating was 1.3 log units above threshold. Varying the adapting spatial frequency did not seem to change the appearance of the aftereffect, but only affected its duration. The aftereffect is thus obtained only by adapting to patterns below about 4 cycles per deg. This conclusion is reinforced by magnitude estimates⁷ of the initial vividness of the aftereffect. The 0.38 cycles per deg adapting pattern produced the most vivid aftereffect, and vividness decreased as the adapting pattern was raised to 3 cycles per deg.

We next measured the effect of temporal frequency with the adapting pattern at 0.38 cycles per deg. Figure 1b shows that the aftereffect is greatest at the highest rates used, 8 and 16 Hz. The adapting and test rates were the same. Very weak effects were obtained when the adapting pattern was 8 Hz and the test

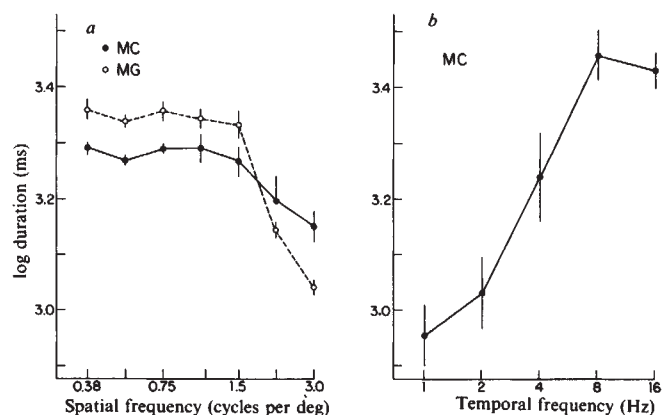


Fig. 1 Duration (± 1 s.d.) of flicker MAE—the rapid motion seen on a uniform flickering field after adapting to a rightward-moving grating. In a, the spatial frequency of the adapting grating was varied. The rate of motion of the adapting grating and the flicker rate of the test field was 8 Hz. Adapting and test stimuli were set 1.3 log units above threshold. Observers M.C. and M.G. adapted to the moving grating for periods of 15 and 30 s, respectively. In b, the moving adapting grating was held at 0.38 cycle per deg, and the rate of motion of the adapting grating was varied. The adapting and test rates were the same. Stimuli were 1.3 log units above threshold, and the adapting period was 90 s.

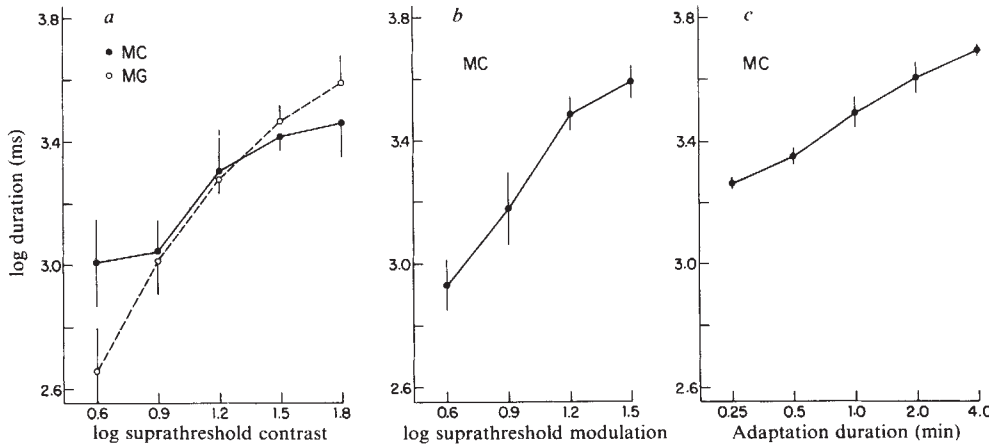


Fig. 2 Duration of flicker MAE. The moving adapting grating was 0.38 cycle per deg; the adapting and test rates were 8 Hz. In *a*, the contrast of the adapting grating was varied. The adapting period was 15 s, and the test flicker was 1.1 log units above threshold. In *b* the amplitude of the test flicker was varied. The adapting period was 90 s and the adapting grating was 1.8 log units above threshold. In *c*, the duration of the adapting period was varied. Adapting and test patterns were 1.3 log units above threshold.

1 Hz or vice versa. Thus, for a strong aftereffect, rapid rates are needed in both adapting and test phases.

Further measurements examined the effect of stimulus contrast. The optimum adapting spatial frequency (0.38 cycle per deg) and adapting and test rates (8 Hz) were used. Figure 2*a* shows that the aftereffect increases as the adapting contrast is raised to the highest level used, 64 times the threshold. Figure 2*b* shows that the aftereffect also increases with the amplitude of the test flicker. Magnitude estimations for two observers showed that the initial vividness of the aftereffect was greater when the test flicker was 1.5 log units above threshold as opposed to 1.2 log units. This result was obtained with adapting spatial frequencies from 0.38 to 3 cycles per deg. The increasing strength of the aftereffect with high test amplitudes is surprising as the traditional MAE is strongest with low test contrasts⁸.

The flicker MAE is also distinguished from the traditional MAE by its duration. The latter may last 20 s⁹, several minutes¹⁰ or longer¹¹. The flicker MAE is quite ephemeral. Long adaptation periods were used to maximize the aftereffect. The aftereffect lasted less than 5 s after 4 min adaptation (Fig. 2*c*), and the effect was extended to only 7 s with 10 min adaptation (data not shown).

We were unable to obtain interocular transfer of the flicker MAE when the moving adapting grating was presented to one eye and the flickering test field was presented to the other eye with a mirror haploscope and two CRTs. Both the flicker and traditional MAEs were seen, however, when the adapted eye simultaneously viewed a stationary grating and uniform flickering field. The stationary grating appeared to drift slowly, and the flickering component contained rapid motion. When the same test stimulus was presented to the unadapted eye, only the slow drift was seen. It is well known that the traditional MAE transfers interocularly³. The flicker MAE transfers poorly if at all.

The most interesting feature of the flicker MAE is its tuning to low spatial frequencies (below 4 cycles per deg). The traditional MAE can be obtained with much finer adapting patterns of 8 or 10 cycles per deg^{12,13}, and the direction-selective threshold elevation is present at 15 cycles per deg¹⁴. The flicker MAE shows that the direction-selective mechanisms tuned to low spatial frequency may respond to uniform flicker. This hypothesis is supported by the finding that adaptation to uniform flicker impairs detection of moving gratings only below 4 cycles per deg^{15,16} and moving gratings below 4 cycles per deg mask uniform flicker¹⁷. Studies^{18,19} on threshold summation between low spatial frequency patterns in rapid flicker show that transient, low spatial frequency mechanisms have high sensitivity to uniform flicker. King-Smith and Kulikowski¹⁸ suggest that such mechanisms may be primarily concerned with the detection of motion. There are presumably also motion analysers sensitive to higher spatial frequencies, as demonstrated by the traditional MAE. Various investigators have obtained psychophysical evidence for several types of motion-processing mechanisms²⁰⁻²³. Murray *et al.*²⁴ hypothesize that two specific classes of mechanisms detect motion: fast motion,

transient mechanisms that are sensitive to low spatial frequencies and receive Y-cell inputs and slow motion, pattern mechanisms that are sensitive to higher spatial frequencies and receive X-cell inputs.

The flicker MAE shows that the lowest spatial frequency motion mechanisms are sensitive to uniform flicker and are directionally selective. An intriguing question is whether the majority of mechanisms sensitive to uniform flicker are highly directionally selective. Directional selectivity provides rich information and thus provides an economy of neural encoding.

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Pioneer axons lose directed growth after selective killing of guidepost cells

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The first nerve cells to appear in the limb buds of embryonic grasshoppers are a pair which lie at the distal tip and project axons along the length of the limb to the central nervous system (CNS)¹. The stereotyped route navigated by these 'pioneer' axons is followed by other neurones and eventually becomes that of a major adult nerve trunk². The guidance cues which delineate this route are unknown, but it has been suggested that guidance is provided by a set of nonadjacent 'guidepost' cells along which the pioneers grow (Fig. 1)^{1,3-6}. We have now tested this suggestion by selectively destroying identified guidepost cells and observing pioneer axon trajectories in their absence. Our results support the guidepost cell hypothesis.

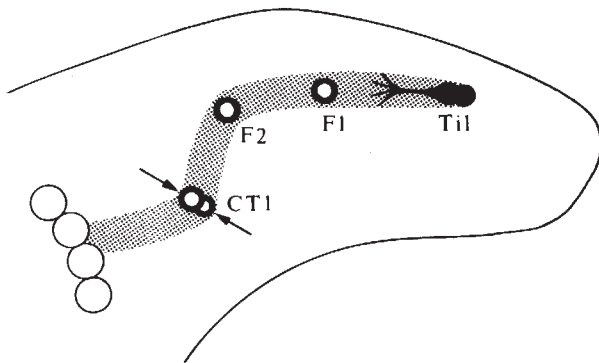


Fig. 1 Guidepost cell hypothesis and experimental design. The pioneer axon route (stippling) extends along a set of distinctive cells (F1, F2, CT1) and then to the CNS. The hypothesis that these cells are the guidance cues for pioneers was tested by destruction of the most proximal guideposts (arrows) when axonogenesis was beginning in the T11 pioneers. The routes taken by pioneers in the absence of guideposts were observed.

At the 30% stage⁷ of embryonic development, the first neurones (designated T11) in the leg arise from a cell within the limb epithelium and migrate into the interior⁸. Their pioneer axons trace a distinctive route to the CNS: they grow first along the anterior margin of the limb (Fig. 2a), cross abruptly to the posterior side (Fig. 2b) and extend to the margin of the CNS (Fig. 2c). Axons of later-arising neurones fasciculate with the pioneers, so that the route of pioneer navigation is preserved as the course of an adult nerve. We are concerned here with the guidance cues for this route.

Examination by scanning and transmission electron microscopy (TEM) of the substrate under nascent pioneer axons reveals no continuous morphological feature which could provide guidance^{3,9}. However, at three sites, cells (designated F1, F2, CT1; Fig. 1) are located which have the following features: (1) the cells lie within the pioneer route^{3,4}; (2) they are distinctive from all surrounding cells (insect neurone-specific anti-horseradish peroxidase (HRP) antibodies¹⁰ bind to these immature neurones³, as do other, monoclonal, antibodies⁴); (3) they are successively contacted by pioneer axon growth cones (with direct membrane apposition confirmed in TEM⁵), so that the sharp turns in the pioneer axons occur as they grow from cell to cell; (4) they form specialized, dye-passing junctions with the pioneer axons^{3,9} (and the pioneers do not form such junctions with any other cells along their pathway); (5) they are sites for re-convergence of the two pioneer axons, which occasionally take separate routes across the epithelium between cells¹¹, indicating that the pioneer guidance substrate is not continuous. The observations suggest that these cells are the guidance cues for pioneer axons (which may locate their guideposts by filopodial exploration^{3-6,8}), and that placement of these cells determines the stereotyped pioneer route.

We have tested this hypothesis by identifying guidepost cells before the pioneer growth cones have reached them, destroying selected guidepost cells by irradiation, culturing the embryos to a stage at which the pioneer axons should have reached the CNS, and comparing the routes which were taken by pioneer axons in the presence and absence of guidepost cells.

As the pioneer axons normally change course sharply to cross to the guidepost cells, designated CT1 (Fig. 2b), destruction of the CT1 cells provides the best test of the hypothesis. However, unlike the pioneers, the CT1 cells are not readily identifiable by morphology. An *in vivo* antibody-labelling technique enabled these cells to be located (Fig. 3; left inset): embryos at the 31% stage of development, when the pioneer axons are just starting to grow, were removed from the egg and an incision was made in the dorsal closure membrane over the thorax to provide access for antibodies to the interior of the limb lumen. The embryos were immersed in saline⁹ containing 1% rabbit-derived fluorescein-conjugated anti-HRP antibodies (Cappel) for 3 h, washed in saline and viewed under a Zeiss epifluores-

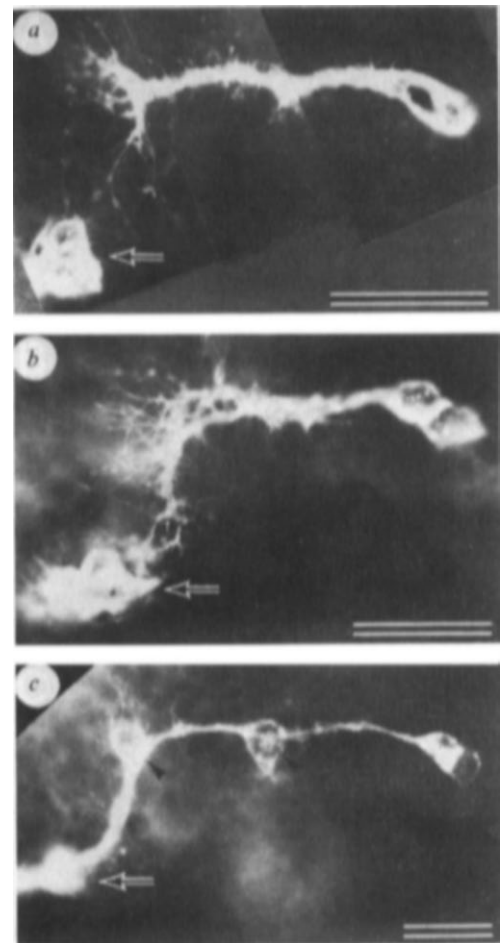


Fig. 2 Normal pathfinding by pioneer neurones. *a*, Pioneer axons extending towards the CNS (left), with the growth cones at the site of cell F2 (not yet staining). Although the axons have not begun to turn towards the CT1 cells (arrow), filopodia from the pioneer growth cones are already in contact with, or very close to, CT1 cell processes. *b*, Pioneer axons turning sharply towards the CT1 cells (arrow). Note the numerous filopodia. *c*, Pioneer axons growing over the CT1 cells (arrow) and continuing towards the CNS. The two characteristic turns in the pioneer pathway have been completed. Note that the F2 and F1 cells (arrowheads) are now staining. Scale bars, 50 μ m (*a*, *b*, prothoracic leg; *c*, metathoracic leg; staining: indirect-labelling with fluorescently tagged antibody in fixed tissue).

cence microscope. At this stage, two CT1 cells stain in the prothoracic leg (Fig. 2a), one stains in the mesothoracic leg (Fig. 3, left inset) and none stains in the metathoracic leg.

In experimental limbs (with matched control contralateral limbs), the CT1 cells were irradiated for 10 min with a beam from a mercury arc-source (Zeiss HB050W; unfiltered other than through the microscope optics). Following irradiation, the cells displayed changes characteristic of cell death, including swelling, cytoplasmic and nuclear granulation, occasional rupture, autofluorescence and selective uptake of trypan blue dye. When examined after the period of embryo culture, irradiated CT1 cells appeared as contracted bodies devoid of processes and with reduced internal structure.

With an iris-diaphragm, the irradiating beam was constricted to a circular profile about 9 μ m in diameter which could be superimposed over the target cell body. Because the target cell was stained with a fluorophore which bleaches rapidly under irradiation, and because small processes of the target cell extended outside the diameter of the iris-diaphragm, localized bleaching was used to confirm that irradiation had not spread beyond the perimeter of the diaphragm. Irradiation of cells lying above and below the target cell was minimized by focusing the beam with a high numerical aperture objective (Leitz

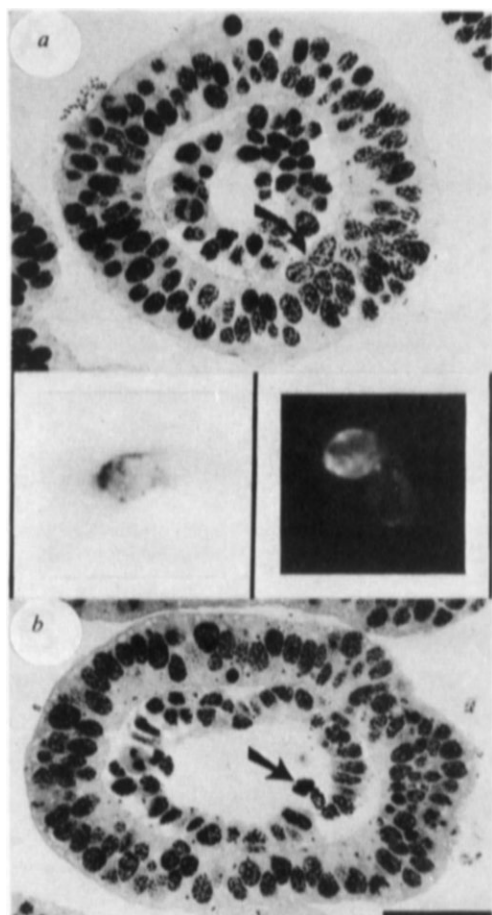
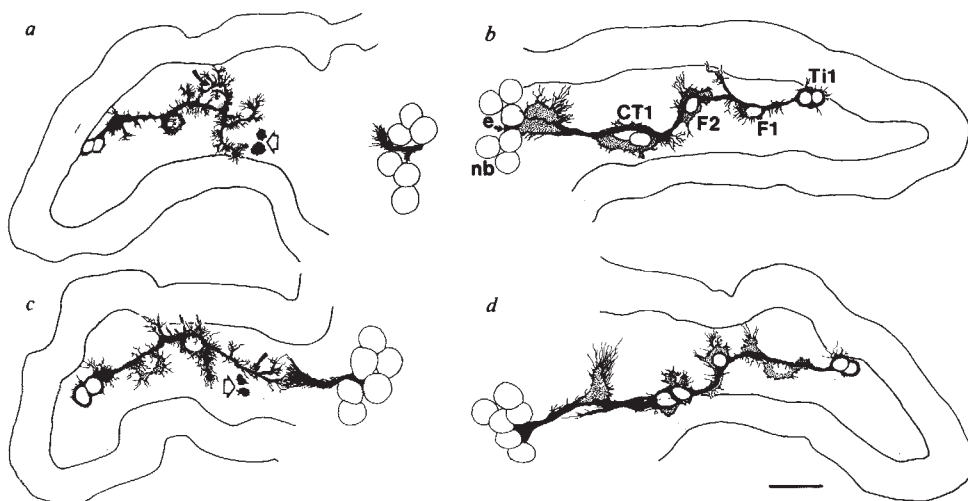


Fig. 3 Irradiation of *in vivo* guidepost cells. *a*, Two CT1 cells (arrow) in a normal leg. The cells lie against the inner surface of the epithelium. Note the inner ring of mesodermal cells. For microbeam targeting, the CT1 cells are selectively stained in live embryos with fluorescently-tagged primary anti-HRP antibody (left inset; epifluorescent illumination; single cell). *b*, Appearance in transmitted light of a pair of CT1 cells (arrow) 1½ h after irradiation. The cells are shrunken, internally disorganized and displaced from the epithelium. Note that the epithelium remains intact, with a continuous internal surface at the CT1 site. These same cells have been identified under epifluorescent illumination (right inset) after indirect-labelling with a fluorescently-tagged antibody. Scale bar, 50 µm (15 µm for insets; all cells are mesothoracic; toluidine blue counterstain).

Fig. 4 Pioneer neurones and guidepost cells in experimental and control limbs. *a*, Experimental leg (camera lucida tracing after antibody indirect-labelling in fixed tissue). Irradiated CT1 cells (open arrow) appear as condensed bodies without processes. The pioneers do not project axons along the normal route, and instead show multiple abnormal branches, with a prominent branch (solid arrow) growing distally. *b*, Contralateral control limb for *a*. A continuous neural path from the pioneer (Ti1) cell bodies to the CNS is established, with the normal pioneer cross-over from F2 to CT1 (nb, central neuroblasts; e, efferent axons emerging from CNS). *c*, Experimental leg with condensed CT1 cells (open arrow). The pioneers do not project axons along their normal route, and have multiple abnormal branches. A prominent branch (solid arrow) has extended proximally along an abnormal route, and has contacted filopodia of efferent growth cones. *d*, Contralateral control limb for *c*. A continuous neural path from the pioneer cell bodies to the CNS is established, with the normal pioneer cross-over from F2 to CT1. All legs are prothoracic and illustrate the range of results observed. Scale bar, 100 µm.



Fluorescenz 50 W, 1.0 n.a.) in the equatorial plane of the target cell.

To assess the damage caused by this technique, embryos were fixed in 4% paraformaldehyde 1½ h after the irradiation, post-fixed in 2% osmium, re-stained with an immunofluorescent indirect-labelling technique (see below), embedded in Araldite, and sectioned at 4 µm. The CT1 cells were identified under epifluorescent illumination (Fig. 3, right inset) and the sections were then counterstained with toluidine blue and examined with transmitted light (Fig. 3). Irradiated CT1 cells were shrunken, displaced from the epithelium and had lost internal structure (Fig. 3b). The epithelium at the site of irradiation was continuous, and had a smooth internal surface (Fig. 3b). Similar results were seen in tissue fixed 24 h after irradiation. We conclude that the irradiation technique did selectively destroy the CT1 cells, and did not cause extensive damage to the overlying epithelium.

After irradiation, experimental embryos were cultured at 30 °C in a hanging drop of either saline⁹, or prepared medium (Schneider's *Drosophila* medium, revised, with L-glutamine); drops were supplemented by sucrose, Gibco antibiotic-antimycotic and, in most cases, freshly-dissected yolk cells. Cultures were maintained until leg morphology, particularly size and segmentation, indicated that the pioneers should have reached the CNS (usually 30–48 h).

After the culture period, embryos were re-stained using an indirect-labelling antibody technique¹²: immersion in rabbit-derived anti-HRP primary antibody (0.1%; 12 h; Cappel) was followed by a quench/wash in normal goat serum (1 h), and then incubation for 2 h in 0.1% fluorescein- or rhodamine-conjugated goat-derived anti-rabbit secondary antibody (Antibodies Incorporated). Stained pioneer axons were traced at ×800 with a camera lucida.

We report the results from examination of 28 limbs (9 experimental/culture-control limb-pairs, and 5 irradiation-control/culture-control limb-pairs) which satisfied the following criteria: pre-irradiation staining confirmed that the pioneer growth cones had not reached cell F2, and that the CT1 cells could be positively identified; embryos developed normal morphology in culture; re-staining demonstrated that in culture-control limbs, a normal continuous axonal pathway from the pioneer cell bodies to the CNS had been established (Fig. 4b, d); and in experimental limbs, the CT1 cells had been successfully irradiated, and the courses of pioneer axons could be seen.

Pioneer neurones in every experimental limb failed to navigate the normal route to the CNS. Moreover, in every case these pioneers did not contact the CT1 cells, had multiple abnormal branches and had at least one prominent branch

which grew along a distinctly abnormal route. The specific pattern of branches varied greatly between individuals. In some cases (five of nine), none of the pioneer axon branches projected more proximally than the CT1 cell site (Fig. 4a); in other cases, a branch did extend past the CT1 site, but on an abnormal route, and in two of these cases, branches made contact with emerging filopodia from efferent neurones (Fig. 4c).

In five irradiation-control embryos, we irradiated a site on the same plane as the CT1 cells, but about 1–2 cell-diameters distal; the pioneer axons normally cross this area on the way to the CT1 cells. Two of these controls were irradiated before pioneer filopodia had reached the area; in both cases, the pioneers subsequently grew to the CT1 cells and established a normal route. Three control embryos were irradiated at a slightly later stage, when some filopodia had invaded the area of irradiation; pioneers in two of these embryos subsequently established a normal route, while in the third embryo the CT1 cells were not contacted and an abnormal process extended towards the CNS. These controls indicate that the abnormal routes seen without exception after irradiation of the CT1 cells are not due to nonspecific effects of the irradiation.

We conclude that after selective destruction of the CT1 cells, the pioneer axons are no longer able to navigate their normal route to the CNS. The variety of pioneer axon configurations seen in these circum stances also indicates that no other guidance cue can substitute for the CT1 cells. Consequently, these results support the hypothesis that the guidepost cells are cues for pioneer neurone pathfinding and that their placement in the limb is essential for complete specification of the pioneer route. This mechanism allows discontinuously distributed substrate cues to guide the establishment of a major, continuous, long-distance nerve route along a specific path.

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Specific binding of ^3H -tiotidine to histamine H_2 receptors in guinea pig cerebral cortex

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Although the H_2 subclass of histamine receptor has been revealed by classical pharmacological approaches^{1,2}, the direct identification of this adenylate cyclase-linked receptor has, despite much effort, remained elusive. Initial studies using ^3H -metiamide and ^3H -histamine^{3,4} and, subsequently, work from our own laboratory and others using ^3H -cimetidine^{5–8} and ^3H -ranitidine⁹ in various tissues, has shown the unsuitability of these ligands for labelling the H_2 receptor. We report here our results using ^3H -tiotidine, a more potent H_2 -antagonist than either cimetidine or ranitidine¹⁰, and show that this ligand meets the criteria for labelling the H_2 receptor in guinea pig cerebral cortex membranes.

Figure 1 shows the binding of ^3H -tiotidine to homogenates of guinea pig cerebral cortex. The specific binding was calculated by subtracting the binding of ^3H -tiotidine in the presence of 5×10^{-3} M histamine from the total binding in the absence of histamine. The specific binding of this H_2 antagonist was saturable and of relatively high affinity. By Scatchard and Hill analysis of three separate experiments the dissociation constant (K_D) and B_{max} values were calculated to be 17 ± 1 nM and 105 ± 12 fmol per mg protein, respectively, and the Hill coefficient was 1.06 ± 0.17 (mean $\pm$ s.d.). Specific binding was linearly related to protein concentration over the range 0.4–2.8 mg ml⁻¹ (final concentration). Throughout the investigation the tissue was used at a final protein concentration of 2 mg ml⁻¹. Specific binding was destroyed after heat-treating the protein in a boiling water bath for 10 min and <0.3% of the ^3H -tiotidine bound to Whatman GF/B filters in the absence of tissue.

The association rate constant (k_1) for specific ^3H -tiotidine binding was 0.0116 ± 0.003 nM min⁻¹ and the dissociation rate constant (k_2) was 0.1227 ± 0.043 , giving a kinetically derived K_D (k_2/k_1) of 10.6 nM.

To establish that ^3H -tiotidine does specifically label the H_2 receptor, we examined the inhibition of ^3H -tiotidine binding to guinea pig cerebral cortex membranes by several known H_2 agonists and antagonists, including the more recently reported compounds YM 11170 (ref. 11) and BL 6341A (ref. 12) (Fig. 2, Table 1). The maximal inhibition of ^3H -tiotidine binding by each compound examined did not differ significantly from that obtained in the presence of 5×10^{-3} M histamine, that is, there was no displacement of the nonspecific component. The non-specific binding routinely accounted for 50–60% of the total binding.

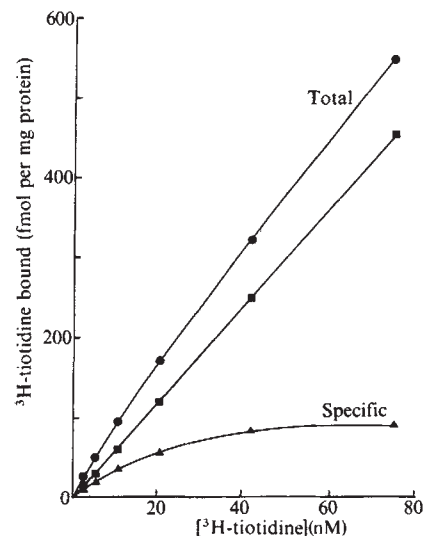


Fig. 1 Binding of ^3H -tiotidine to guinea pig cerebral cortex. Cerebral cortex membranes were prepared essentially as described by Rising *et al.*²¹. The cortex was removed and homogenized in 50 mM sodium-potassium phosphate buffer, pH 7.4, using a Potter homogenizer and centrifuged at 50,000g for 10 min in a Sorvall RC-2B centrifuge. The resulting pellet was washed three times by resuspending in phosphate buffer followed by recentrifugation. The pellet was finally resuspended in phosphate buffer, pH 7.4 at a protein concentration of 5 mg ml⁻¹. Homogenate (100 μ l) was incubated with varying concentrations of ^3H -tiotidine in the presence (■) or absence (●) of 5×10^{-3} M histamine for 30 min at 20 ± 2 °C in a total volume of 250 μ l. The reaction was stopped by adding 2 ml of ice-cold phosphate buffer and the mixture filtered under reduced pressure onto Whatman GF/B glass-fibre filters, followed by three 3-ml washes with room temperature buffer. Radioactivity was determined by allowing the filters to stand for at least 18 h in NE 260 scintillator, followed by liquid scintillation counting. Values are the mean of triplicate determinations. The difference between all pairs of points was statistically significant ($P \leq 0.01$). The ^3H -tiotidine (74.7 Ci mmol⁻¹); was shown by TLC and liquid scintillation counting to be at least 95% radiochemically pure throughout this study. ▲, Specific binding (fmol per mg protein).

Table 1 Inhibition constants (K_i) for ^3H -tiotidine binding

Compound	K_i (M)	Slope
BL 6341A	$1.0 \pm 0.5 \times 10^{-8}$	0.55 ± 0.06
YM 11170	$1.2 \pm 1.4 \times 10^{-8}$	0.88 ± 0.21
Tiotidine*	$2.5 \pm 1.8 \times 10^{-8}$	1.22 ± 0.32
Tiotidine	$3.4 \pm 2.8 \times 10^{-8}$	0.98 ± 0.12
Ranitidine	$3.9 \pm 1.2 \times 10^{-7}$	0.94 ± 0.14
Cimetidine	$4.7 \pm 3.1 \times 10^{-7}$	0.88 ± 0.05
Metiamide	$5.1 \pm 3.0 \times 10^{-7}$	1.05 ± 0.48
Burimamide	$3.0 \pm 0.5 \times 10^{-6}$	0.69 ± 0.12
SKF 91581	$1.6 \pm 0.6 \times 10^{-4}$	1.00 ± 0.08
Impromidine	$6.3 \pm 3.4 \times 10^{-8}$	1.06 ± 0.22
Histamine	$4.3 \pm 3.3 \times 10^{-5}$	0.88 ± 0.26
Dimaprit	$4.4 \pm 3.3 \times 10^{-5}$	0.82 ± 0.10
4-Methylhistamine	$2.7 \pm 0.8 \times 10^{-4}$	0.88 ± 0.32

The K_i values and slopes were calculated from Hill plots and results are expressed as mean $\pm$ s.d. where $n = 3-4$. ^3H -tiotidine was used at 2 nM except for one set of experiments (marked by an asterisk) when ^3H -tiotidine at 16 nM was used. The following non- H_2 compounds caused $<20\%$ inhibition at 5×10^{-5} M: mepyramine, triprolidine, 2-methylhistamine, scopolamine, noradrenaline, 5-hydroxytryptamine, atropine and clonidine.

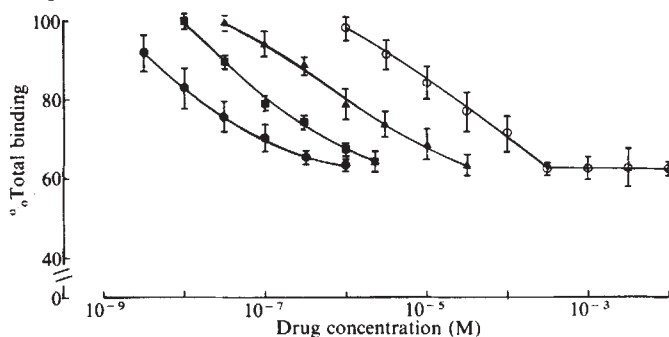


Fig. 2 Inhibition of ^3H -tiotidine binding. Incubations were as described in Fig. 1 legend with 2 nM ^3H -tiotidine and varying concentrations of the competing drug in triplicate. The inhibition constant (K_i) was calculated from the relationship

$$K_i = \text{IC}_{50} / (1 + [T]K_D^{-1})$$

where IC_{50} is the concentration of the drug required for 50% inhibition of specific ^3H -tiotidine binding, $[T]$ is the concentration of ^3H -tiotidine in the assay and K_D the dissociation constant for ^3H -tiotidine, taken as 17 nM. ●, YM 11170; ■, tiotidine; ▲, cimetidine; ○, histamine.

We compared the inhibition constants as calculated from ^3H -tiotidine binding studies with the K_B values obtained from more classical biochemical (adenylate cyclase from guinea pig gastric mucosa) and pharmacological (chronotropic activity in guinea pig right atrium) methods for establishing histamine H_2 specificity (for results see Table 2).

As far as we are aware this is the first report of binding studies on the histamine H_2 receptor that meets the criteria for receptor binding, as initial studies using ^3H -cimetidine had showed this ligand to label an imidazole recognition site⁵⁻⁸. Binding of ^3H -cimetidine could not be displaced by tiotidine or ranitidine, both non-imidazole-containing H_2 antagonists, or by dimaprit, a non-imidazole agonist. Similarly, ^3H -ranitidine binding could not be displaced by cimetidine, tiotidine, dimaprit or histamine⁹. In addition, Batzri *et al.*¹³ have recently used ^3H -histamine to characterize the H_2 receptor on isolated gastric cells, but have failed to show a correlation between binding constants and those obtained by other pharmacological procedures.

We have shown that ^3H -tiotidine binds to a single saturable component, with a dissociation constant similar to that derived from classical pharmacological studies. Its binding to guinea pig cerebral cortex membranes is inhibited by several known H_2 agonists and antagonists of diverse structure. For the H_2 antagonists the correlation between ^3H -tiotidine binding and both adenylate cyclase and guinea pig right atrium activity was excellent ($r = 0.99$, $P < 0.001$) for both systems (see Table 2).

Table 2 Comparison of the activity of H_2 antagonists and agonists

Compound	Tiotidine binding K_i^* (M)	Adenylate cyclase (gastric mucosa) K_B (M)	Right atrium K_B (M)
BL 6341A	1.0×10^{-8}		
YM 11170	1.2×10^{-8}	$5.2 \pm 1.3 \times 10^{-9}$	$3.5 \pm 0.8 \times 10^{-8}$
Tiotidine	3.4×10^{-8}	$2.0 \pm 0.9 \times 10^{-8}$	$3.7 \pm 0.9 \times 10^{-8}$
Ranitidine	3.9×10^{-7}	$2.0 \pm 0.7 \times 10^{-7}$	$1.2 \pm 0.4 \times 10^{-7}$
Cimetidine	4.7×10^{-7}	$7.9 \pm 1.9 \times 10^{-7}$	$4.8 \pm 1.2 \times 10^{-7}$
Metiamide	5.1×10^{-7}	$5.5 \pm 2.1 \times 10^{-7}$	$8.5 \pm 1.9 \times 10^{-7}$
Burimamide	3.0×10^{-6}	$3.2 \pm 1.7 \times 10^{-6}$	$7.4 \pm 0.7 \times 10^{-6}$
SKF91581	1.6×10^{-4}	$7.2 \pm 2.1 \times 10^{-5}$	$2.1 \pm 1.1 \times 10^{-4}$
Impromidine	6.3×10^{-8}	6.5×10^{-8}	1.5×10^{-8}
Histamine	4.3×10^{-5}	1.2×10^{-6}	9.5×10^{-7}
Dimaprit	4.4×10^{-5}	6.0×10^{-6}	5.9×10^{-6}
4-Methylhistamine	2.7×10^{-4}	3.0×10^{-6}	2.5×10^{-6}

The K_i values for ^3H -tiotidine binding were calculated as in Table 1. Adenylate cyclase was measured using the method of Hegstrand *et al.*¹⁷. Aliquots (120 μg) of fundic mucosal homogenate were incubated in 100 mM Tris buffer, pH 7.8, containing 0.6 EGTA, 1 mM isobutylmethyl xanthine (IBMX), 2 mM MgCl_2 , 0.1 mM GTP, histamine at varying concentrations between 0.1 μM and 100 μM and the compound under investigation at varying concentrations between 0.1 μM and 1 mM, for 10 min at 0°C . The reaction was initiated by addition of ATP (1 mM) followed by a 10 min incubation at 30°C . The reaction was stopped by placing the assay tubes in a boiling water bath for 3 min. After cooling, 40 mg of Alumina 90 was added before mixing and centrifugation at 700g for 15 min at 4°C . The cyclic AMP content of each sample was determined using the method of Brown *et al.*¹⁸ using cyclic AMP in a competitive protein binding assay and the K_B values (mean $\pm$ s.d., $n = 3$) determined from Schild plots¹⁹. Activity in the isolated guinea pig right atrium was determined using the method of Reinhardt *et al.*²⁰. The right atrium of the guinea pig heart was dissected free and suspended under a tension of 0.8–1.0 g in an organ bath containing Krebs–Henseleit solution aerated with 5% CO_2 in oxygen. The chronotropic effect, induced by histamine at concentrations between 1 nM and 10 mM, was measured via an isotonic transducer connected to a ratemeter and a potentiometric recorder. Antagonists were used at concentrations necessary to cause a shift to the right in the dose-response curve for histamine. The K_B values (mean $\pm$ s.d., $n = 3$) were determined from Schild plots¹⁹.

* s.d. values as in Table 1.

However, the lack of a similar correlation for the H_2 agonists is not surprising as inhibition of ^3H -tiotidine binding is an index of the affinity of a compound for the receptor, whereas agonist 50% effective concentration (EC_{50}) is additionally dependent on agonist efficacy and the extent of the receptor reserve. This is in accord with Stephenson's proposal¹⁴ that the affinity and the efficacy of an agonist are independent of one another.

Unlike Maayani *et al.*¹⁵, using rat hippocampus, we have shown that ^3H -tiotidine binding to guinea pig cerebral cortex is heat-labile and is not due to filter binding. Reports that other workers (Maayani *et al.*¹⁵ and Batzri *et al.*¹⁶) have failed to demonstrate specific ^3H -tiotidine binding may be due to a combination of factors. Initial work in our laboratory using ^3H -tiotidine showed the ligand to be unstable. In fact, early batches, in our hands, were approximately 50% pure and the results from binding experiments were consequently very variable. Later batches of ligand, as reported here, were stable with a radiochemical purity $>95\%$ by TLC and liquid scintillation counting. Using the method described here we have so far been unable, to demonstrate specific ^3H -tiotidine binding to other tissues, notably guinea pig gastric mucosa and right atrium, and rat uterus and cerebral cortex.

This is the first report of a binding study on the H_2 receptor which correctly ranks the activity of the known H_2 agonists and antagonists and which can discriminate between closely related H_1 and H_2 active compounds (for example, displacement by 4-methylhistamine and very little displacement by 2-methylhistamine). The observation that compound BL 6341A has a Hill slope significantly different from 1, in contrast to the remaining compounds tested, is in agreement with our findings in the guinea pig right atrium, where it was shown to be a non-competitive antagonist of histamine. For this reason no K_B values could be calculated, either from this or the adenylate cyclase system.

Finally, the selectivity of ^3H -tiotidine for the H_2 receptor is shown by the lack of significant inhibition by several non- H_2 compounds (Table 1).

These results suggest that ^3H -tiotidine should be a valuable tool for investigating and further characterizing the histamine H_2 receptor.

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Malignant conversion of mouse skin tumours is increased by tumour initiators and unaffected by tumour promoters

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Multi-stage carcinogenesis (initiation-promotion) was first demonstrated in mouse skin^{1,2}. The first stage, initiation, is accomplished by a low dose of carcinogen that causes no tumours. Promotion by repeated treatment of initiated mice with certain non-carcinogenic hyperplastic agents results in the rapid production of numerous benign papillomas, a few of which progress to squamous cell carcinomas. Although this model system produces mostly benign tumours, many of the concepts concerning carcinogenesis in epithelial tissues have been derived from mouse skin studies. The permanent change in growth potential accomplished by tumour initiators is generally considered to be a mutagenic event³; cell selection and clonal expansion of initiated cells may be involved in promotion⁴. In initiation-promotion experiments, more than 90% of the squamous cell carcinomas develop from papillomas^{5,6}, but the conversion rate is low. The factors necessary for this conversion of benign to malignant tumours have not been defined but tumour promoters have been assumed to be involved. However, we report here that the tumour promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is ineffective in the conversion of papillomas to carcinomas whereas three initiators, urethane, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 4-nitroquinoline-*N*-oxide (4-NQO) are effective. This suggests that malignant conversion may result from a further genetic change in papilloma cells and that the ineffectiveness of TPA may be due to its inactivity as a mutagen.

A tumour induction experiment designed to test the effects of various agents (tumour initiators and promoters) on the rate of conversion of papillomas to carcinomas requires a large

number of papillomas per mouse and a level of carcinomas in which an increase or decrease could be detected. Previous experiments with Sencar mice, bred for sensitivity to initiation-promotion tumorigenesis, had established doses of 7,12-dimethylbenz(a)anthracene (DMBA) as initiator and TPA as promoter as well as the duration of promotion necessary to meet these requirements⁷.

Papillomas were induced in Sencar mice by initiation with 20 µg DMBA (stage I) followed by 10 weeks of promotion (stage II) with TPA (2.5 µg per 0.2 ml acetone topically once a week). Groups of 30 papilloma-bearing animals were either treated with the solvent acetone, continued on TPA treatment or treated weekly with MNNG (120 µg per 0.2 ml acetone topically), urethane (20 mg per 0.2 ml saline intraperitoneally, i.p.) or 4-NQO (250 µg per 0.2 ml acetone topically) on weeks 11-40 (stage III). Papillomas and suspected carcinomas were counted weekly. The diagnosis of squamous cell carcinoma was confirmed histologically at the time each tumour-bearing mouse was killed or at the conclusion of the experiment at week 52.

Stage I (20 µg DMBA) and stage II (2.5 µg TPA weekly) treatment produced over 30 papillomas per mouse with a peak incidence at 16 weeks. Continued promotion with TPA for 20 or 30 weeks did not alter this papilloma incidence. Stage III treatment with TPA produced no significant increase in carcinoma formation compared with the acetone-treated control group (Fig. 1). In both groups, carcinomas developed in 30-35% of the mice by week 40, with a final carcinoma yield of 46-50% at 52 weeks. In contrast, carcinomas appeared earlier

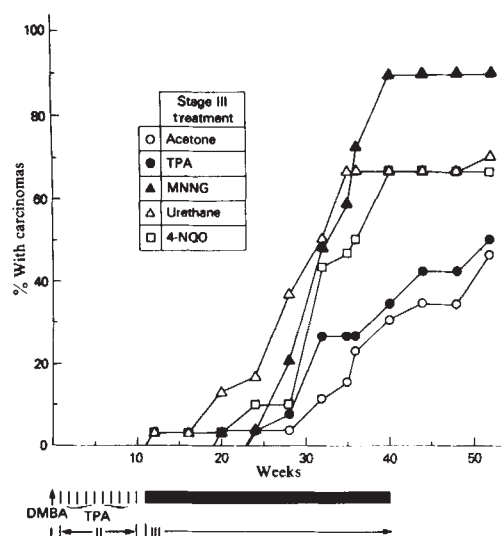


Fig. 1 A comparison of carcinoma development in papilloma-bearing mice treated in stage III with acetone, TPA, MNNG, urethane or 4-NQO. Tumours were induced in groups of 30 7-8-week-old female Sencar mice by the treatment schedule indicated at the bottom of the figure. Stage I initiation by a single topical application of DMBA (20 µg per 0.2 ml acetone) was followed by stage II promotion with TPA (2.5 µg per 0.2 ml acetone weekly for 10 weeks) in all groups. Weekly stage III treatments during weeks 11-40 were as follows: topical application of 0.2 ml acetone (○), or 0.2 ml acetone containing 2.5 µg TPA (●), 120 µg MNNG (▲) or 250 µg 4-NQO (□) or i.p. injection of 20 mg urethane per 0.2 ml saline (△). Papillomas and suspected carcinomas were counted weekly. The '% with carcinomas', calculated as the number of carcinoma-bearing mice divided by the number of mice alive in each group when the first carcinoma appeared, and expressed as a percentage, is plotted against weeks of the experiment. Statistical evaluation of the data was based on methods for comparing onset rates of carcinomas observed in a 'mortality-independent context'¹⁸. The log rank test¹⁹, which adjusts for intercurrent mortality, was used to compare the time of occurrence of the first carcinoma in each animal in the various groups. Two-tailed *P* values obtained by this procedure are as follows for stage III treatments: TPA versus acetone, 0.8397; MNNG versus acetone, 0.0001; urethane versus acetone, <0.0001; 4-NQO versus acetone, 0.0055.

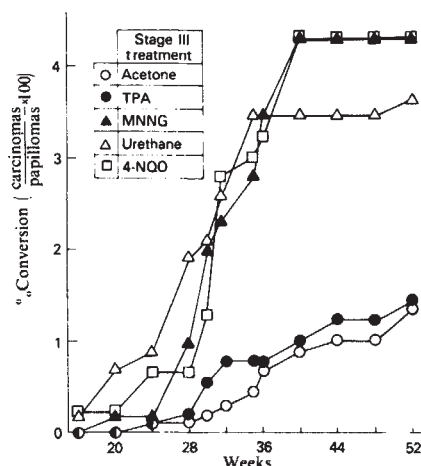


Fig. 2 A comparison of the rate of malignant conversion in groups of papilloma-bearing mice treated in stage III with acetone, TPA, MNNG, urethane or 4-NQO. From the results shown in Fig. 1 and the papilloma yield in each group (not shown), the rate of malignant conversion was calculated. The number of animals which developed one or more carcinomas within 52 weeks was divided by the maximum yield of papillomas in each group, and expressed as a percentage. Two-tailed *P* values obtained by the Fisher-Irwin exact test¹⁹ for comparing the malignant conversion at 52 weeks in each group with the acetone-treated control are as follows: TPA, 0.420; MNNG, 0.0001; urethane, 0.002; 4-NQO, 0.005. A statistically significant difference (*P* < 0.05) from the acetone control group was first seen at week 20 for urethane, week 28 for MNNG and week 30 for 4-NQO.

and in greater numbers in the three groups treated with tumour initiators during stage III. With MNNG, urethane or 4-NQO, maximum carcinoma yields of 67–90% were attained by week 40 (Fig. 1). The rate of carcinoma development in these three groups was significantly different from that found in the groups treated in stage III with acetone or TPA.

As most carcinomas develop from papillomas, the rate of malignant conversion may be calculated as the number of carcinomas divided by the maximum number of papillomas. The results of these calculations are shown in Fig. 2, in which the conversion rate is expressed as a percentage. After the development of one or two carcinomas, mice died or were killed when moribund or at 52 weeks for histological verification of suspected malignant tumours. For the calculation of malignant conversion rate, only the first squamous cell carcinoma on each animal was counted. Thus, the per cent conversion does not indicate the full potential of conversion of papillomas to carcinomas because only the first malignant tumour on each animal is counted. However, the value calculated is meaningful for comparison of the stage III treatments.

With solvent treatment in stage III, 1.34% of the papillomas progressed to carcinomas by week 52. With stage III TPA treatment, the rate of malignant conversion was 1.45%. There is no statistical difference between the conversion rates for these two groups. Stage III treatment with urethane shortened the latent period for carcinoma formation by 12 weeks and increased the malignant conversion rate to 3.6%. With stage III treatment with either MNNG or 4-NQO, the rate of malignant conversion was increased to 4.3%. Both agents reduced the latent period by about 8 weeks. The rates of malignant conversion for the three initiators were all statistically different from TPA or acetone, but not different from each other.

The papilloma and carcinoma incidence in control groups treated with the solvent acetone in stage I are shown in Table 1. Following acetone treatment in stage II, stage III injections of urethane were not tumorigenic. 4-NQO was weakly tumorigenic, producing a few papillomas (0.36 per mouse) but no carcinomas. MNNG was moderately active as a complete carcinogen, inducing three papillomas per mouse and four carcinomas in the 30-animal group. TPA treatment in stage II,

Table 1 Tumour development in non-initiated mice treated with acetone in stage I

Stage III treatment	Stage II treatment			
	Acetone		TPA	
	Papillomas per mouse (max.)	Mice with carcinomas (%)	Papillomas per mouse (max.)	Mice with carcinomas (%)
Acetone	—	—	0.56	1 (4.8)
TPA	—	—	0.77	1 (4.2)
MNNG	3.0	4 (13.8)	4.1	5 (20)
Urethane	0	0 (0)	1.04	4 (16)
4-NQO	0.36	0 (0)	0.15	1 (5.6)

followed by stage III application of acetone, produced a maximum papilloma incidence of 0.56 per animal (14 papillomas on 25 mice surviving at week 24), with one carcinoma (Table 1). This tumour induction in non-initiated Sencar mice treated with TPA has been observed previously⁷. Continued TPA treatment in stage III did not increase the papilloma or carcinoma incidence. In the group treated with TPA in stage II and MNNG in stage III, the number of tumours approximated the summation of the two treatments. This was not the case with 4-NQO, where the papilloma incidence was less than that found for either TPA or 4-NQO alone, but one carcinoma developed. When stage III urethane injections followed stage II TPA treatment, the papilloma incidence was doubled and four mice developed carcinomas. This unexpected co-carcinogenic response may be related to the large increase in initiation by urethane when an application of tumour promoter preceded a single urethane treatment⁸. In all control groups, the carcinoma yield was small, indicating the marked increase in risk associated with the presence of multiple papillomas.

Earlier literature reports suggested to us the probable relevance of the present experiment. Squamous cell carcinomas can be induced by repeated treatment with initiating doses of DMBA. This protocol produces fewer papillomas but many more carcinomas than the initiation-promotion protocol⁹. This finding led Shubik¹⁰ to suggest that "the changes necessary to... influence the rate of malignant transformation seem to be identical with the change involved in initiation". More recently, Scribner and Scribner¹¹ have drawn similar conclusions from studies using 7-bromomethylbenz(a)anthracene as a complete carcinogen or as a tumour promoter. The effectiveness of complete carcinogens in the induction of malignant tumour formation is contrasted with the ineffectiveness or even inhibition of carcinoma formation by TPA^{6,12}. On the other hand, results from cell culture studies¹³ suggest that TPA can accomplish the final irreversible step of *in vitro* transformation, but the relevance of this model system to *in vivo* epidermal carcinogenesis is unclear.

In contrast to the apparent inability of TPA to enhance malignant conversion *in vivo*, Roe *et al.*¹⁴ reported in a preliminary study that treatment with the pure initiator urethane after papillomas have formed increased their conversion to carcinomas. Other lines of evidence have led to the suggestion that malignant tumour formation may result from two or more carcinogen-induced mutations and that the role of promotion in carcinogenesis is to enlarge the size of the target cell population available for the second mutation^{15–17}.

We have shown that a relatively short period of TPA treatment is effective in the promotion of benign papillomas after DMBA initiation, but that continued TPA treatment does not affect the progression of papillomas to carcinomas. A single mutation, perhaps in a gene controlling the response of cells to signals to differentiate terminally, may be sufficient for initiation¹⁷. The mechanism of TPA selection for the growth of initiated cells in papilloma formation is unclear, but the inability of initiated cells to respond to promoter-induced terminal differentiation may be a critical factor¹⁷. We have shown that three tumour initiators significantly increase the rate of

malignant conversion. The genotoxic nature of these agents suggests that the induction of malignant tumours may require two (or more) mutations.

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Papillomas induced by initiation-promotion differ from those induced by carcinogen alone

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The discovery of a two-stage mechanism of carcinogenesis in mouse skin, with initiation and promotion as independent components, provided new approaches to the study of the development of neoplasms in experimental animals and humans¹⁻⁵. However, it is not clear how the carcinogen and promoting agent are involved at different steps in the development of papillomas and carcinomas. Here we have used cell markers in mice to study the mechanism of development of papillomas induced by the classical method of two-stage skin tumorigenesis (initiation with subtumorigenic doses of a carcinogen followed by promotion with phorbol esters) and by multiple treatments with initiating doses of a carcinogen. Our results show that papillomas induced by repeated carcinogen applications arise from significantly more cells than those induced by the carcinogen-promoter regimen.

We have used X-chromosome-linked phosphoglycerate kinase (PGK) isoenzymes as markers to determine the number of cells from which skin papillomas develop. Because of inactivation of one of the two X chromosomes in each somatic cell in female mice during embryogenesis, only one of the two PGK genes is active in any single somatic cell. Thus, mice heterozygous for the usual gene (*Pgk-1^b*) and the variant *Pgk-1^a* have two cell populations, one producing PGK B and the other, variant-type PGK A. In such animals, neoplasms with a unicellular origin should exhibit only a single-enzyme phenotype (A or B), whereas those with a multicellular origin might exhibit double-enzyme phenotypes (both A and B). By using these PGK heterozygous mice, we and others have shown that high doses of methylcholanthrene in olive oil or tricaprilin, when injected subcutaneously, can induce fibrosarcomas of multicellular origin^{6,7}.

In the present experiments, we found no tumours in skins of mice after application of 10 µg of tetradecanoyl phorbol acetate (TPA) three times a week for 25 weeks or after a single application of 200 µg of 7,12-dimethylbenz(a)anthracene (DMBA). In contrast, a single application of 200 µg of DMBA followed by repeated TPA (10 µg) promotion three times a week induced papillomas in 85% of mice, with a mean of 6.4 papillomas per mouse (Table 1). The average time of tumour appearance in 50% of the mice (*t*₅₀) was 14 weeks. Most of the papillomas (88%) regressed after termination of TPA promotion, with a mean of 0.8 tumours per mouse remaining after 10 weeks. As anticipated, normal tissues obtained from the mice at autopsy exhibited equal proportions of PGK B and A isoenzyme activities. In contrast, 86% of the 83 skin papillomas exhibited single-enzyme PGK phenotypes. Increasing the dose of DMBA to 800 µg resulted in a shorter *t*₅₀, but no significant increase in the frequency of papillomas with double-enzyme PGK phenotypes or in the number of papillomas per mouse (Table 1). The fact that most skin papillomas that developed after TPA promotion of mouse skin initiated with a single application of DMBA had single-enzyme phenotypes suggested strongly that most of the neoplasms had developed from one or a relatively few cells.

In another experiment, we studied papillomas that arose after repeated sequential applications of DMBA in the absence of a promoter. Although a single dose of 200 µg of DMBA did not induce tumours, repeated applications with this dose for 14 weeks induced papillomas in 95% of mice. These papillomas, however, differed from those which resulted from the standard initiation-promotion regimen in several respects. For example, they had more double-enzyme PGK phenotypes (46% of 147 versus 14% of 83 papillomas) (*P* < 0.01) and fewer of them regressed (58% versus 88%, *P* < 0.001). The difference in PGK phenotypes suggests that sequential exposure to 200 µg of DMBA alone without TPA promotion induced papillomas which arose from more cells than papillomas induced with a single application of DMBA followed by TPA promotion.

Table 1 Effect of increasing doses of DMBA on papilloma formation

DMBA (µg)	Mice No. with tumours/no. treated	<i>t</i> ₅₀ (wk)	Papillomas per mouse		Papilloma PGK phenotypes	
			Average no.	Range	No. tested	% Double-enzyme
200	17/20	14	6.4	0-8	83	14
400	10/10	8	9.0	2-12	45	22
800	10/10	8	8.5	2-11	46	20

BALB/c PGK heterozygous (*Pgk-1^b/Pgk-1^a*) mice from the seventh to tenth backcross generation exhibiting PGK B/A ratios of 1:1 in blood cells were used in all experiments. The required concentration of DMBA (Sigma) or TPA (P.L. Biochemicals, Milwaukee) was applied topically in 0.2 ml spectroscopic acetone (Baker Chemicals, Seattle). DMBA was applied once and was followed by application of 10 µg TPA three times a week for 20 weeks. The backs of mice were shaved at least 72 h before initial topical DMBA treatment. TPA promotion was started 48 h after initiation with DMBA. All mice were examined three times a week for papilloma formation. Papillomas of >2 mm diameter were counted for determination of tumour incidence and those of >5 mm size were used for PGK tests. They were dissected free of normal tissues by cutting them slightly above the elevated or pedunculated base. PGK phenotypes of tumours and normal tissues were determined by starch-gel electrophoresis as described previously⁶. The relative activity of the PGK isoenzymes was estimated visually using known mixtures as standards. With this technique we can detect a minor population in a mixture of cells if it contributes 2-5% of the total PGK activity. The ratios of B/A enzyme activities in normal tissues such as skin, blood, liver, kidney, heart and thigh muscle were determined in all mice after termination of the experiment. The skin was further separated into dermis and epidermis by treating it with trypsin.

Table 2 Effect of TPA applications on papilloma formation induced by repeated applications of DMBA

TPA	Mice No. with tumours/no. treated	t_{50}	Papillomas per mouse		Papilloma PGK phenotypes	
			Average no.	Range	No. tested	% Double-enzyme
No	19/20	9.5	9.0	0-16	147	46*
Yes	10/10	6.0	19.0†	9-23	55	18

All animals received weekly applications of 200 μ g DMBA. Those not receiving TPA had 0.2 ml acetone applied three times a week for 14 weeks; those receiving TPA had 10 μ g TPA in 0.2 ml acetone applied three times a week for 14 weeks.

* $P < 0.01$ (χ^2).

† $P < 0.001$ (Student's t -test).

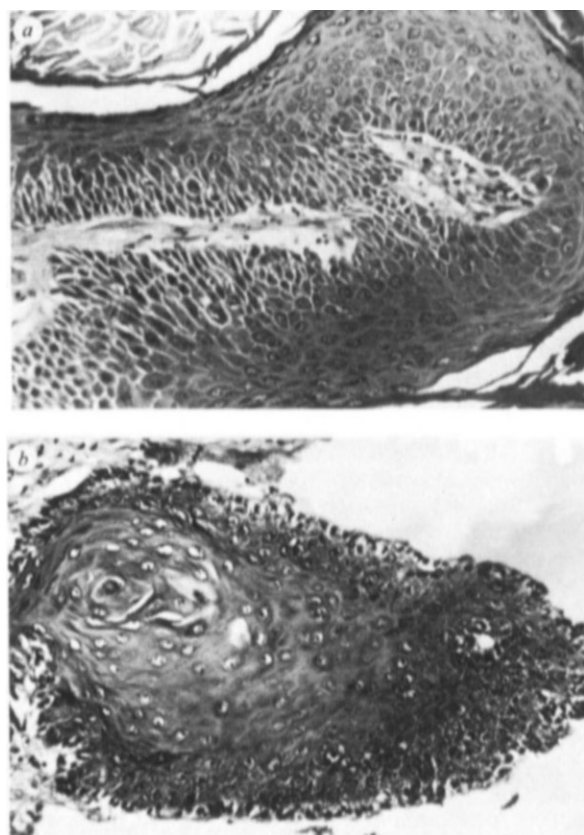


Fig. 1 Skin papillomas. *a*, Tumour induced by a standard initiation-promotion regimen, showing a single-enzyme PGK phenotype. *b*, Tumour induced by 14 weekly applications of DMBA (200 μ g), showing a double-enzyme PGK phenotype. Note the presence of multilayered hyperplastic epithelium and sparse stroma in both papillomas. Haematoxylin and eosin stains used. $\times 320$.

In the final experiment, we tested the effect of a combination of repeated DMBA plus repeated TPA applications on tumorigenesis (Table 2). Papillomas appeared earlier and there were more tumours per animal ($P < 0.001$) and more with single-enzyme PGK phenotypes ($P < 0.01$) in mice receiving repeated DMBA with TPA than in those receiving DMBA alone. Furthermore, 10 weeks after termination of treatment of mice receiving repeated DMBA and TPA, 93% of papillomas had regressed ($P < 0.001$).

Several possibilities other than multicellular origin should be considered for papillomas with double-enzyme PGK phenotypes. For example, a double-enzyme phenotype could be observed in a clonal tumour if it arose from a single cell with both X chromosomes active, if the neoplasm was cross-contaminated with another papilloma or if there was admixture of non-neoplastic cells in the tumour sample tested. If the papillomas with double-enzyme phenotypes arose from a single cell with both chromosomes active, equal amounts of PGK B and A isoenzyme activities would be expected. However, the ratio of PGK B/A activity varied from papilloma to papilloma

and rarely was 1:1. Cross-contamination with other papillomas is unlikely because all tumours were separated from each other without any physical evidence of coalescence and biopsies were always performed at early stages in tumour development.

To estimate admixture with non-neoplastic cells, histopathological studies were performed on randomly selected large papillomas exhibiting single- or double-enzyme PGK phenotypes. Tissues were fixed in 10% formalin in phosphate-buffered saline (pH 7.2) and sections were stained with haematoxylin and eosin. The per cent of tumour and nontumour cells was estimated without knowledge of the results of isoenzyme analyses. The histological examinations provided no evidence of significant contamination with non-neoplastic cells. No differences were observed between tumours showing single- and double-enzyme PGK phenotypes (Fig. 1). Both types of papilloma were found to contain hyperplastic squamous epithelium at least 8-12 layers thick surrounding a sparse stroma containing very few cells. Thus, it is most likely that the papillomas which exhibited double-enzyme phenotypes originated from more than one cell, that is, they were multiclonal in origin.

The finding of a single-enzyme phenotype in a tumour does not necessarily indicate that the neoplasm arose from a single cell. It might have developed from several cells which had the same PGK isoenzyme. This could happen in tissues where daughter cells remain adjacent to one another and form patches during development. The probability of multiclonal tumours exhibiting single-enzyme phenotypes will increase as the patch size increases. To evaluate patch size, we cut dermal and epidermal tissues obtained by trypsinization of skin from papilloma-bearing mice into small fragments. The smallest fragment we could obtain and test was approximately 3 mm². Such samples consistently exhibited equal amounts of PGK A and B activities, suggesting good mixing of cells in tissues (that is, small patch sizes) in PGK mosaic mice. Although these data do not exclude the possibility that neoplasms with single-enzyme phenotypes arose from a few cells of identical PGK type, it can certainly be concluded that they arose from fewer cells than those which exhibited double-enzyme phenotypes.

Thus, the observation that more papillomas had double-enzyme PGK phenotypes when DMBA was applied repeatedly in the absence of TPA promotion suggests strongly a difference in the number of cells from which the tumours developed in the two groups—papillomas induced by repeated applications of DMBA in the presence of TPA arose from fewer cells than papillomas induced by the same dose of DMBA in the absence of promoter. This conclusion supports the postulate that papillomas induced by an initiation-promotion regimen arise by a qualitatively different mechanism from those induced with complete carcinogens⁸. Other findings supporting this postulate are: initiation-promotion-induced papillomas do not have autonomous growth and very few of them develop into carcinomas compared with those induced by repeated applications of carcinogen^{9,10}; and retinoids inhibit formation of papillomas induced with TPA promotion but not those induced by DMBA applications alone^{11,12}.

The possibility that the promoter acts on a subpopulation of DMBA-altered cells which would not develop into papillomas in the absence of promotion is supported by our observation that fewer papillomas (more likely to appear multicellular in origin) developed in the group treated with DMBA alone. The

promoting effect of TPA need not necessarily be through a direct action on DMBA-altered cells. For example, TPA might block the transmission of growth signals to normal cells or enhance the transmission of such signals to DMBA-altered cells. The observation that many papillomas regress when TPA applications cease is consistent with the suggestion that TPA allows the expression of a 'neoplastic' phenotype in DMBA-treated cells by an epigenetic mechanism.

Finally, the experiments reported here demonstrate the use of mice with X-chromosome-linked PGK markers for studies of the early stages of neoplastic development and the mechanisms of tumour initiation and promotion.

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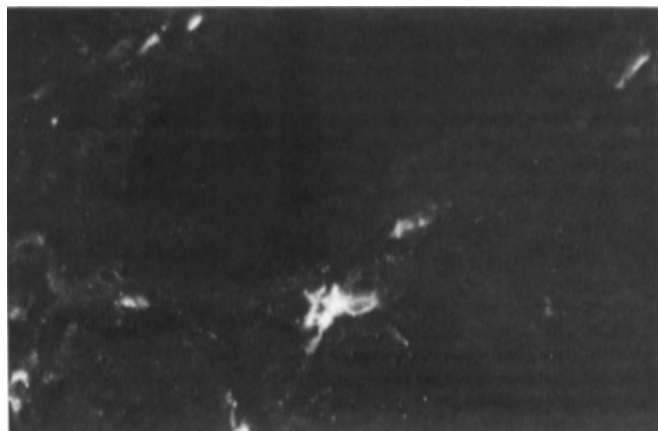


Fig. 1 Unfixed cryostat section of normal human thyroid gland stained by indirect immunofluorescence with anti-DR monoclonal antibodies $\times 200$. DA2 (ref. 6), 2.06 (ref. 7), MID-3 and control monoclonal antibodies were applied at $10 \mu\text{g ml}^{-1}$. FITC-conjugated rabbit anti-mouse immunoglobulin (Dako) previously absorbed with human immunoglobulins linked to Sepharose 4B was used as the second layer. HLA-DR staining is confined to the vascular endothelium and occasional interstitial cells located under the basement follicular membrane. No staining was observed on thyroid epithelial cells.

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Lectin-induced expression of DR antigen on human cultured follicular thyroid cells

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HLA-DR antigens, the human equivalent of mouse *I* region-associated or Ia products, are polymorphic cell surface sialoglycoproteins involved in initiation of the immune response¹. Their expression is normally restricted to B lymphocytes, macrophages, dendritic and other antigen-presenting cells and vascular endothelium² and possibly some cells of the mucosa lining body cavities^{3,4}. HLA-DR expression can be modified during cell differentiation; B lymphocytes become negative on maturing to plasma cells and human T lymphocytes acquire these antigens when activated *in vitro* or *in vivo*⁵. We report here that human thyroid follicular cells which are normally negative for HLA-DR molecules, can be induced to express these antigens when cultured with phytohaemagglutinin (PHA), concanavalin A (Con A) or pokeweed mitogen (PWM). These lectins exert their action directly on the thyroid cells with no concomitant mitogenic effect.

DA2 (ref. 6), 2.06 (ref. 7) and MID-3 (G. Guarnotta, P. Lydyard and P. Banga, unpublished) mouse monoclonal antibodies to the nonpolymorphic part of the DR molecule were used in conjunction with the indirect immunofluorescence technique. Two additional mouse monoclonal antibodies to ox red blood cells (P. Lydyard, unpublished) and mouse thyroglobulin⁸ which do not react with human tissues, provided the controls (see legends to Figs 1, 2). Cryostat sections, freshly dispersed viable cell suspensions and monolayer cultures prepared from normal human thyroids were studied. On frozen sections only

some capillary endothelial and a few scattered interstitial cells were stained while follicular epithelial cells were negative for DR (Fig. 1). In cell suspensions no membrane fluorescence was visible on the follicular cells and they remained negative when cultured as monolayers for up to 9 days (Fig. 2a, b). When PHA at $0.86 \mu\text{g ml}^{-1}$, equivalent to 10^{-2} mitogenic units per ml (purified PHA; Wellcome), was added to the culture medium a high percentage of thyroid cells became positive for DR on the membrane (Fig. 2c, d). Although this effect was already detectable at 24 h, the proportion of positive cells and the brightness of the fluorescence increased until day 8 of culture in most of the glands used (Fig. 3). No staining was observed on the thyroid cells when control antibodies were used instead of DA2, 2.06 or MID3.

To further ascertain the identity of these cells the same cultures were incubated with the serum of a patient with Hashimoto's disease known to contain antibodies against thyroid surface ('microvillar') determinants⁹. The human and mouse antibodies were detected by the addition in turn of fluoresceinated and rhodaminated specific antisera. Although red and green fluorescence was observed on the same cells, the two sets of antigens (HLA-DR and microvillar) were clearly distinct entities expressed on the surface of the thyroid cells. Similar expression of DR could also be induced by PWM (1:400 to 1:40; Gibco, batch R293012) and Con A ($20 \mu\text{g ml}^{-1}$; ICN Pharmaceuticals) although the fluorescence was less intense than that observed after culturing the cells with PHA. In contrast, no DR expression was elicited by the addition of pituitary thyrotropin ($50 \mu\text{U ml}^{-1}$ to 50 mU ml^{-1} ; Armour) to the thyroid cultures at 24 h. Incubation with PHA did not produce an apparent increase in the number of thyroid cells.

To confirm the lack of mitogenic effect of PHA on these cells, monolayers were pulsed with ³H-thymidine at 3, 4, 5, 7 and 9 days of culture. The radioisotope uptake observed in these conditions, however, was no higher than that measured in control thyroid cultures. In addition, PHA did not stimulate *de novo* expression of HLA-DR molecules in similar cultures of 18-week-old human fetal thyroid and fibroblast cells. Negative immunofluorescence staining was also obtained in PHA-stimulated cultured rat thyroid cells using monoclonal anti-rat RT-1B (Ia-like antigens) (MAS-029, Sera Lab).

To exclude the possibility that the surface DR was passively acquired from contaminating lymphocytes, the cultures were

Fig. 2 Phase-contrast (*a, c*) and indirect immunofluorescence micrographs (*b, d*) of 6-day-old monolayer cultures of normal thyroid cells stained with mouse monoclonal anti-DR antigen. In the control monolayer only macrophages were strongly positive (*b*) while after culture with PHA, thyroid follicular cells showed a bright fine granular membrane fluorescence due to the presence of HLA-DR antigens (*d*) $\times 500$. The staining of the same cultures with control monoclonals (of the same immunoglobulin subclass) was completely negative and only the macrophages had a coarse granular fluorescence on the membrane due to nonspecific binding of immunoglobulins to the Fc receptors of these cells. Normal thyroid tissue obtained at surgery was digested with collagenase type IV (Worthington) 5 mg ml^{-1} for 3 h at 37°C . The digest was filtered through a $200\text{-}\mu\text{m}$ mesh and cells were plated at a concentration of 10^5 per ml onto 13 mm glass coverslips in a Linbro 24-well plate. RPMI 1640 culture medium (Flow) was supplemented with 2 mM glutamine, $8 \text{ }\mu\text{g ml}^{-1}$ insulin (Novo), $5 \text{ }\mu\text{g ml}^{-1}$ transferrin (Sigma), 10^{-8} M hydrocortisone (Sigma) and 20% fetal calf serum (Gibco). At 24 h cultures were washed to remove unattached cells and re-fed with culture medium containing PHA at concentrations ranging from 10^{-4} to 5×10^{-2} mitogenic units per ml. Viable monolayers were stained on the coverslips from day 1 to day 8 after addition of PHA. Cultures were washed before and after incubations with Hank's balanced salt solution containing 1% bovine serum albumin and incubated with $75 \text{ }\mu\text{l}$ of mouse monoclonal antibody prediluted ($10 \text{ }\mu\text{g ml}^{-1}$) at room temperature for 30 min followed by a similar incubation with $75 \text{ }\mu\text{l}$ FITC-rabbit anti-mouse immunoglobulin conjugate preabsorbed with human immunoglobulin and diluted 1:20. Finally, the monolayers were fixed with 5% acetic acid in ethanol at -20°C for 10 min, mounted in glycerol and viewed under a Zeiss UV microscope. To identify the positive cells double-label indirect immunofluorescence was used. Cultures were sequentially incubated with prediluted patient's serum containing thyroid microvillar antibodies followed by rabbit anti-human immunoglobulin-rhodamine conjugate (Miles). Monolayers were then stained for DR expression as described above (not shown).

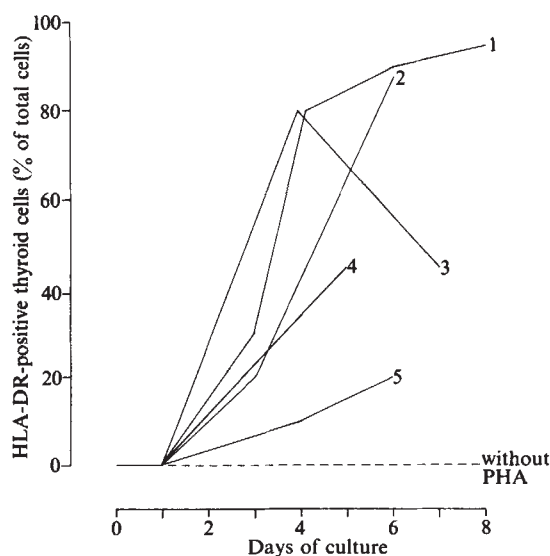
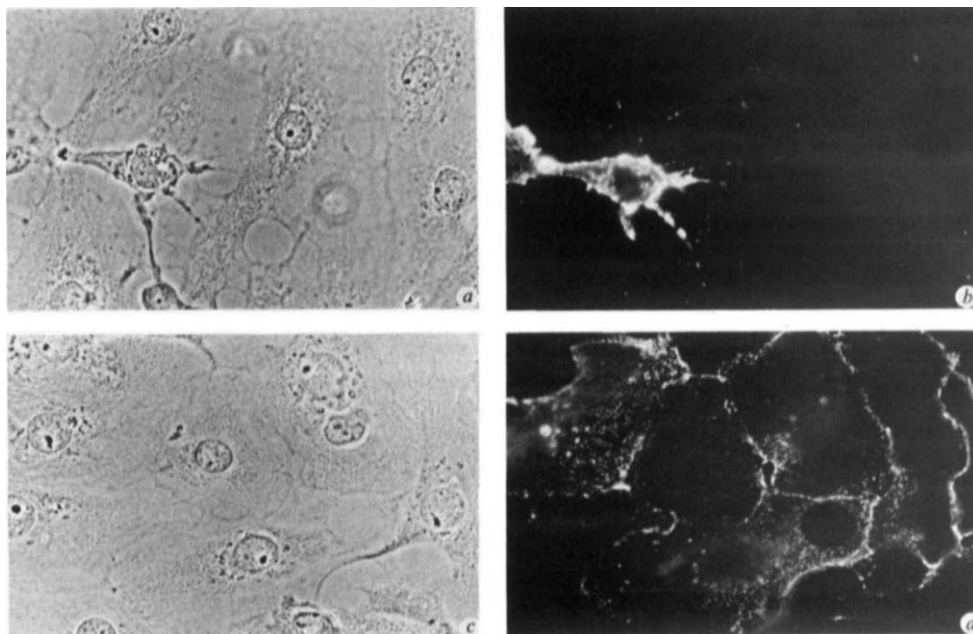


Fig. 3 Time-related effect of PHA (10^{-2} mitogenic units per ml) on DR antigen expression in thyroid monolayer cultures obtained from five different normal glands. DR antigen was first detected after 24 h. The intensity of fluorescence and the number of positive cells increased gradually in most cases. Thyroids 2 and 3 were normal glands obtained from patients undergoing radical neck surgery for carcinoma of the larynx while cultures 1, 4 and 5 were taken from peri-adenomatous tissue.

washed repeatedly in order to remove lymphocytes and non-adherent cells. Their depletion was confirmed by immunofluorescence using an anti-human pan-T monoclonal antibody (UCHT1)¹⁰. By the same method, no T cells were detected after treatment of the cultures with the combination of monoclonal antibodies MBG6 (ref. 11) and RFT2 and complement. (G. Janossy, E. Price-Jones, J. Hann, E. Rawlings, L. Trejdosiewicz and M. Bofill, unpublished). Both these antibodies are complement-fixing and cytotoxic for human T cells. In the two cases where these treatments were carried out, the induction of DR expression following PHA stimulation was unaffected which rules out a possible lymphokine-mediated effect of thyroid cells. Furthermore, no DR staining was observed after thyroid cells were preincubated for 2 h with the supernatant derived from PHA-activated normal human lymphocytes. In a different experiment, contaminating macrophages were removed by sequential incubations on plastic for 45 min and with carbonyl-iron particles for 1 h at 37°C . The cells were then plated onto coverslips and stimulated with PHA. DR expression was again unaffected by this procedure. When the same cultures were fixed in acetone and stained, numerous fluorescent-positive vesicles in the perinuclear region were seen, indicating an active synthesis of DR molecules at the cytoplasmic level¹² (Fig. 4).

We previously reported that normal human thyroid follicular cells possess HLA-A, B, C but not DR antigens¹³, as expected. The above experiments however, prove that DR antigen expression on thyroid cells can be induced. The mechanisms of action of PHA and other lectins are not fully understood but DR induction on the thyroid cells is unrelated to the known mitogenic effects of lectins. Neither does this phenomenon seem to be part of a general biological effect as human fetal thyroid fibroblasts and rat thyroid cells failed to express DR after PHA challenge. Modulation of DR antigen expression has been



Fig. 4 Immunofluorescence micrograph of a 7-day-old thyroid monolayer fixed with acetone and stained with mouse monoclonal anti-HLA-DR as described before, showing the presence of DR antigens in cytoplasmic vesicles scattered around the nucleus. $\times 300$.

observed only exceptionally outside the immune system¹⁴; guinea pig mammary gland duct epithelium becomes Ia-positive during pregnancy¹⁵ and lactation. Ia antigen expression can be induced during graft-versus-host disease¹⁶ and in some neoplastic conditions¹⁷. In these cases an immunological role has been postulated for the Ia⁺ cells.

The inducibility of DR expression on thyroid cells is most remarkable in view of autoimmune phenomena affecting this gland. It is now reasonable to postulate that as yet unidentified factors may induce DR expression on thyroid cells *in vivo*. This may be an important initial or additional event by which the presentation of the relevant autoantigens to already programmed autoreactive T helper cells could lead to an effective autoimmune response. Work is in progress to determine whether a similar phenomenon can be demonstrated in other endocrine cells often affected by autoimmune reactions.

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Multiple organ-reactive monoclonal autoantibodies

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Autoantibodies directed against a wide range of normal tissue antigens have been found in the sera of patients with autoimmune diseases¹⁻⁸. It is generally thought that different and specific autoantibodies react with different tissues but the possibility exists that some autoantibodies may react with common antigens found in different tissues and organs. Recently, we showed that mice infected with reovirus developed a polyendocrine disease with autoantibodies to the pancreas, anterior pituitary, thymus and gastric mucosa^{9,10}. Using hybridoma technology, we obtained a number of monoclonal autoantibodies¹¹ which reacted with antigens in single organs. We now report the production and pattern of reactivity of seven multiple organ-reactive monoclonal autoantibodies. By using antibody-affinity columns, autoantigens also have been isolated and their molecular weights determined. The results suggest that monoclonal multiple organ-reactive autoantibodies react either with the same molecule present in several organs or with common antigenic determinants on different molecules in multiple organs. In either case, the existence of multiple organ-reactive antibodies may be a partial explanation for multiple organ autoimmunity.

Spleen cells from SJL/J mice infected with reovirus type 1 were fused with P3-653Ag8 myeloma cells; the culture fluids were screened by indirect immunofluorescence (FA) for antibodies reactive with paraffin sections of Bouin's-fixed normal mouse tissues (for example, adrenal glands, brain, muscle, pancreas, pituitary, salivary glands, skin, stomach, intestine, thymus, liver and thyroid), as previously described¹¹. Hybrids producing autoantibodies were immediately cloned by the limiting dilution method¹². Several days after cloning, each well was microscopically examined to ensure that it contained only a single colony. In addition, when two of the antibodies (MOR-1 and MOR-4) were subjected to isoelectric focusing on gels with a pH gradient of 3.5-9.5, they showed a restricted pattern typical of monoclonal antibodies¹³. Seven stable hybridomas that synthesize multiple organ reactive autoantibodies were isolated from five different animals. The reactivity pattern of these antibodies was determined by indirect immunofluorescence and/or by indirect immunoperoxidase staining using paraffin sections of normal mouse tissues fixed in Bouin's solution. The two methods gave very similar results, but the latter was generally more useful in visualizing structural details and identifying certain cell types¹⁴.

Figure 1 shows the reactivity pattern of one of these autoantibodies (MOR-1). This antibody reacts with cells in the pituitary, pancreas, small intestine and stomach. When incubated with sections of pituitary, MOR-1 stains cells in the anterior, but not the posterior of intermediate lobes of the pituitary (Fig. 1a,b). The identity of cells involved is not known, but they do not appear to be growth hormone- or prolactin-producing cells as evaluated by staining with fluorescein-labelled antibodies to these hormones. MOR-1 also stains cells in pancreatic islets, but not cells in pancreatic acinar tissue

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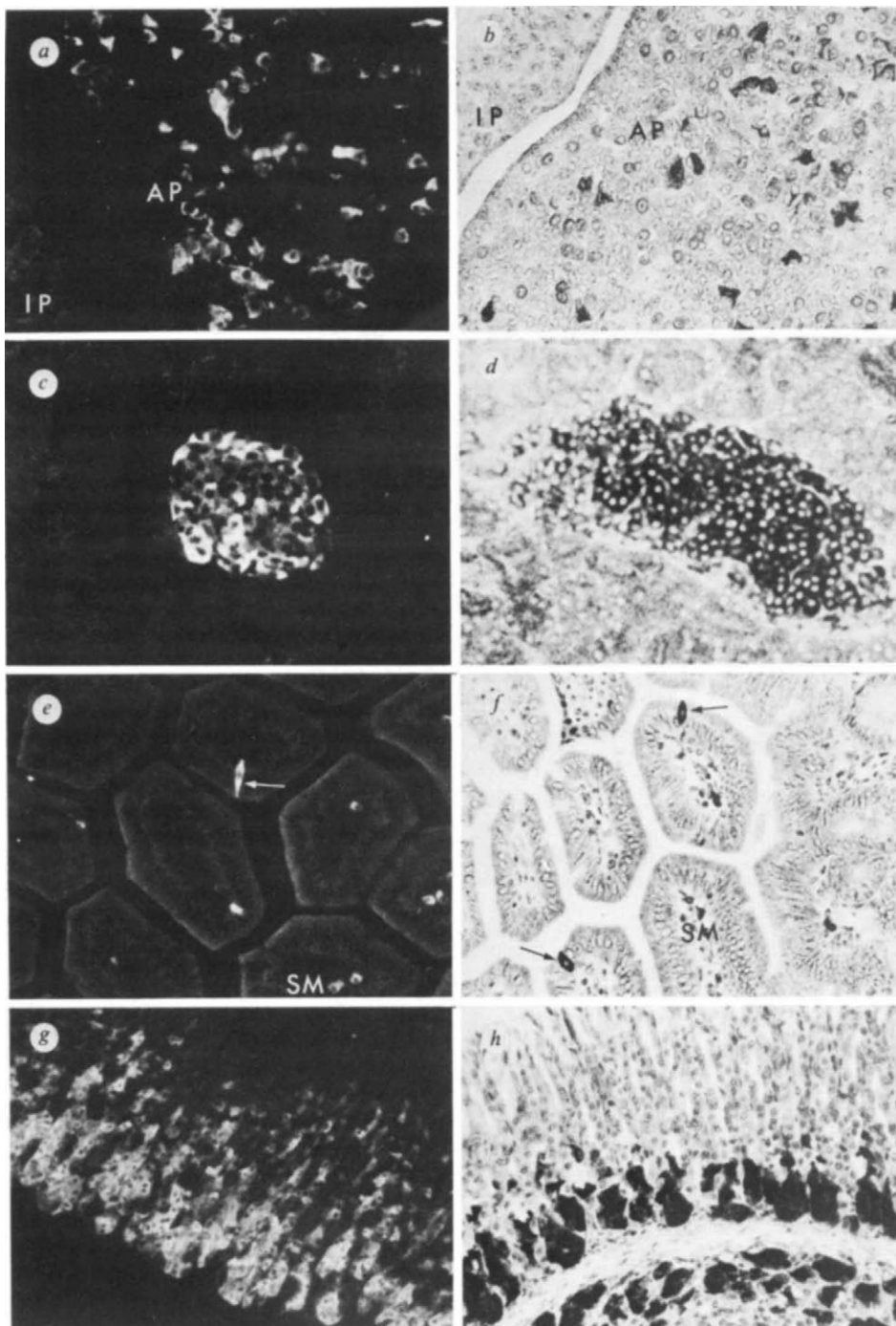


Fig. 1 Monoclonal autoantibody MOR-1 reacts with multiple organs. Normal mouse pituitary, pancreas, small intestine and stomach were fixed for 6 h in freshly prepared Bouin's solution. The specimens were washed in six changes of 70% ethanol over 72 h, embedded in paraffin and sectioned. The deparaffinized sections were first incubated with monoclonal autoantibody MOR-1 and then with affinity purified goat antibody to mouse IgM conjugated with either fluorescein isothiocyanate for indirect immunofluorescence⁹ or peroxidase for indirect immunoperoxidase¹³. Panel on left stained by immunofluorescence; panel on right by immunoperoxidase. *a, b*, Sections of pituitary. Cells in the anterior pituitary (AP) stained with MOR-1. There is no staining of cells in the intermediate (IP) or posterior lobes of the pituitary. Magnifications: *a*, $\times 340$; *b*, $\times 230$. *c, d*, Sections of mouse pancreas showing islet cells, but not acinar cells, stained with MOR-1. Magnifications: *c*, $\times 340$; *d*, $\times 230$. *e, f*, Sections of small intestine. Single isolated cells scattered throughout the small intestine (arrows) stained with MOR-1. The small labelled cells within the submucosa (SM) are most probably IgM containing plasma cells. Magnifications: *e*, $\times 165$; *f*, $\times 140$. *g, h*, Sections of stomach. Population of cells within the gastric mucosa stained with MOR-1. Magnifications: *g*, $\times 165$; *h*, $\times 150$.

(Fig. 1*c, d*). This antibody does not react with glucagon, insulin or growth hormone in enzyme-linked immunosorbent assays (ELISA) (data not shown). MOR-1 also stains single isolated basal granulated cells scattered throughout the small intestine. These cells morphologically resemble enteroendocrine cells (Fig. 1*e, f*). By staining consecutive sections, we observed that these cells are distinct from those reacting with antisera to glucagon, enkephalin and somatostatin (data not shown). MOR-1 also reacts strongly with cells in the gastric mucosa (Fig. 1*g, h*). The stained cells have been identified as chief cells by the presence of pepsinogen granules¹⁵, but absorption of MOR-1 with porcine pepsinogen failed to block the specific labelling of gastric mucosa cells (data not shown).

The reactivity pattern of the seven multiple organ-reactive antibodies is shown in Table 1. MOR-1 reacts with cells in the gastric mucosa, anterior pituitary, small intestine and pancreatic islets; MOR-2-7 do not react with cells in the small intestines,

and MOR-3 and -4 do not react with cells in the pancreatic islets. Considerable differences in the relative FA titres were observed, especially when reactivity with gastric mucosa and anterior pituitary was compared. None of the seven antibodies reacted with thyroid, adrenals, salivary glands, liver, thymus, brain, muscle or cultured mouse embryo fibroblasts (data not shown).

To isolate the autoantigens that react with multiple organ-reactive antibodies, homogenates of whole stomach (St) or pituitary (Pt) were passed through multiple organ-reactive antibody-affinity columns. Antigens St-1 and Pt-1 were eluted from an MOR-1 affinity column and antigen St-4 from an MOR-4 affinity column. These antigens were concentrated and tested on nitrocellulose paper for their reactivity with all seven MOR antibodies. As seen in Table 1, MOR-1 reacted strongly with St-1 and Pt-1, but not with St-4. Conversely, MOR-4 reacted strongly with St-4, but not with St-1 or Pt-1. MOR-2 showed

Table 1 Reactivity pattern of multiple organ-reactive monoclonal autoantibodies

Monoclonal autoantibody	Animal	Fluorescent antibody titre				Reactivity with affinity purified autoantigens		
		Gastric musosa	Anterior pituitary	Small intestine	Pancreatic islets	St-1	Pt-1	St-4
MOR-1	5B	800	800	400	40	+	+	-
MOR-2	12D	100	50	<2	10	±	±	-
MOR-3	13D	400	50	<2	<2	-	-	-
MOR-4	13D	100	100	<2	<2	-	-	+
MOR-5	13E	100	10	<2	10	-	-	-
MOR-6	13E	400	400	<2	20	-	-	-
MOR-7	13F	100	400	<2	10	-	-	-
AP-2	5B	<2	800	ND	<2	ND	ND	ND
GM-1	5A	400	<2	<2	<2	±	-	-

Monoclonal autoantibodies, precipitated from supernatant fluids by 50% saturated ammonium sulphate, were tested for reactivity with normal mouse tissues. Bouin's-fixed paraffin sections were incubated with serial dilutions of the autoantibodies followed by incubation with affinity-purified goat antibody to mouse IgG and IgM conjugated with fluorescein isothiocyanate. The fluorescent antibody titre is the reciprocal of the highest dilution giving positive fluorescence. The immunoglobulin class was determined by Ouchterlony double immunodiffusion. All the antibodies were of the IgM class, except MOR-4 which is IgG2b. To isolate the autoantigens, stomachs or pituitaries from SJL/J mice were minced and washed repeatedly in 0.1 M Tris-HCl containing 0.15 M NaCl (pH 7.8). Following Dounce homogenization, the homogenates were mixed with an equal volume of extraction buffer (0.1 M Tris-HCl, 0.15 M NaCl, 2% NP40) (pH 7.8), rehomogenized and clarified at 100,000g for 60 min. The supernatants thus obtained were filtered (0.2 µm) and passed through a column containing affinity purified monoclonal MOR-1 or MOR-4 autoantibodies covalently linked to cyanogen bromide-activated Sepharose CL-4B. The molecules bound to the monoclonal antibodies were eluted with either 50% ethylene glycol (pH 11.5) (that is MOR-1) or 1 M acetic acid (that is, MOR-4). The eluted material (St-1 and Pt-1 from the MOR-1 column and St-4 from the MOR-4 column) was dialyzed against distilled water and lyophilized. The reconstituted eluate was spotted onto nitrocellulose paper, allowed to dry and then soaked in 3% bovine serum albumin. Following overnight incubation at 4 °C with monoclonal autoantibodies, the spots were incubated for 2 h at room temperature with affinity-purified goat antibody to mouse IgG and IgM conjugated with peroxidase and then reacted with H₂O₂ and diaminobenzidine. The nitrocellulose blots were then examined for brown reaction product. (+) Strongly positive; (±) weakly positive; (-) negative.

weak reactivity with both St-1 and Pt-1. All the other antibodies failed to react with St-1, Pt-1 or St-4. Similar results, especially with MOR-1 and MOR-4, were obtained in an ELISA assay in which the antigens were adsorbed onto microtitre plates and incubated with the seven antibodies (data not shown). Taken together, these experiments show that although these antibodies react by immunofluorescence with cells in the gastric mucosa and anterior pituitary, they are not all recognizing the same antigenic determinants.

To further characterize the autoantigens, concentrated stomach and pituitary eluates from a MOR-4 affinity column were electrophoresed in SDS-polyacrylamide gels (PAGE). Four major bands (molecular weights 25,000 (25K), 43K, 53K and 62K) were obtained with the stomach eluate, and one major band (~25K) with the anterior pituitary eluate (Fig. 2a,b). The latter band consistently showed a slightly faster mobility than the 25K band from the stomach. When the gels of stomach and pituitary antigens were blotted on nitrocellulose paper^{16,17}, MOR-4 reacted with only three (25K, 43K, 53K) of the major stomach bands and the major pituitary band (Fig. 2c,d).

The present work demonstrates that monoclonal autoantibodies can react with antigens in multiple organs. The most likely explanation is that monoclonal autoantibodies are reacting with the same molecule present in several different tissues, and/or that they are reacting with common antigenic determinants on different molecules in multiple organs. Both explanations will probably be found to be true¹⁸. Our data (Fig. 2) also suggest that the multiple organ-reactive antibodies may recognize common determinants on different molecules even within the same organ. Although the autoantigens have not been fully characterized, the fact that multiple organ-reactive autoantibodies react with only certain mouse organs and not with cultured mouse cells (unpublished data) argues that these autoantibodies are not directed against easily recognized cytoskeletal elements¹⁹.

Our studies also suggest that such antibodies are not uncommon. Although initially only seven hybridomas that synthesized multiple organ-reactive autoantibodies were found, a much larger number have recently been identified by extending our screening procedure to include more organs. There also appears to be considerable diversity in the antigenic determi-

nants recognized. Thus, different animals, and in some cases, the same animal, make different autoantibodies to antigens in the same organ. Experiments in progress suggest that some hybridomas obtained from the spleens of normal mice also may synthesize multiple organ-reactive antibodies.

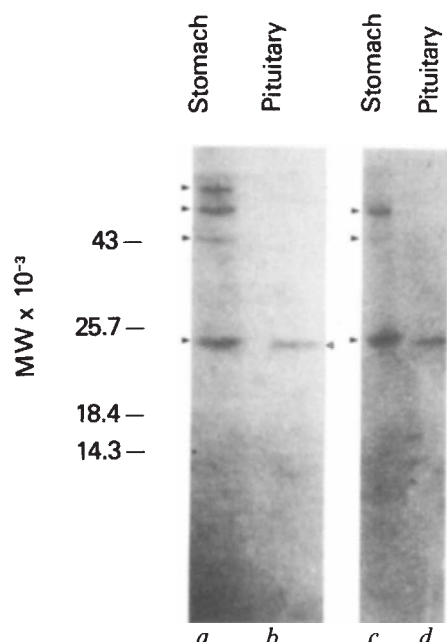


Fig. 2 Reactivity of autoantigens with MOR-4 antibody. Antigens from pituitary and stomach were isolated by affinity chromatography with MOR-4 antibody as described in the legend to Table 1, electrophoresed on 10% SDS-PAGE and blotted onto nitrocellulose paper^{16,17}. Lanes a and b show the Coomassie blue staining of affinity purified stomach and pituitary antigens. Lanes c and d show the Western blots of antigens isolated from stomach and pituitary stained with MOR-4 antibody by the immunoperoxidase method. Solid and open arrows indicate polypeptides isolated from stomach and pituitary, respectively. Numbers represent molecular weight markers.

The studies in mice have been extended to humans. Human-human and human-mouse hybridomas have been prepared from patients with polyendocrine disease. These hybridomas synthesize autoantibodies that react with multiple normal human tissues (for example pituitary, gastric mucosa, pancreas and thyroid)²⁰. Thus these antibodies may be a partial explanation for multiple organ autoimmunity in both animals and humans. A molecule in one organ may share common antigenic determinants with molecules in other organs. An antibody elicited against a common antigenic determinant in one organ would react with all organs containing that determinant, resulting in multiple organ autoimmunity. The availability of large quantities of monoclonal multiple organ-reactive autoantibodies should aid in isolating autoantigens and studying the molecular basis of autoimmunity.

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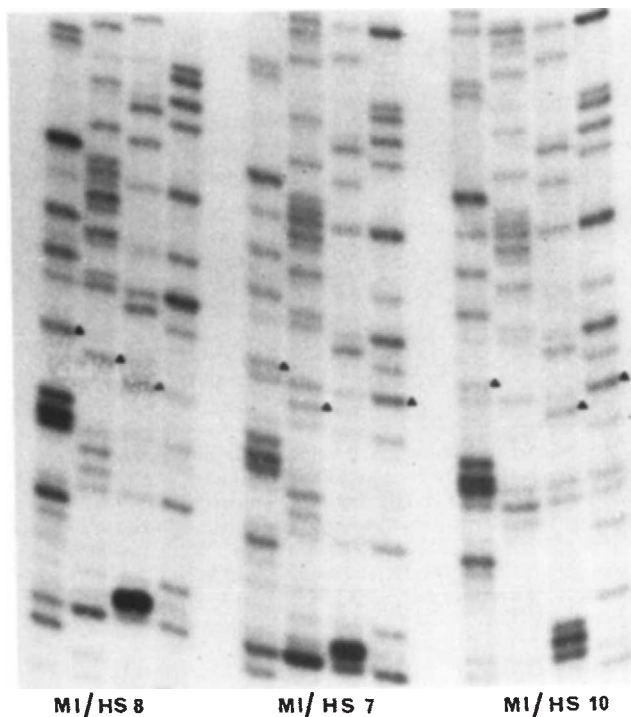


Fig. 1 Nucleotide sequence changes in the RNA of receptor binding variants. Sequences were determined using the dideoxynucleotide chain terminating procedure⁸. Each reaction mixture, 10 μ l, contained Tris-Cl pH 8.3, 0.05 M; magnesium chloride, 0.012 M; dithiothreitol, 0.02 M; dATP, dCTP, dGTP, dTTP, 0.0004 M; human placenta RNase inhibitor (Bethesda Research Labs) 3 units; reverse transcriptase (Life Sciences) 5 units; and ddATP, ddCTP, ddGTP or ddTTP at either 0.00025 M or 0.00006 M and was incubated for 120 min at 42 °C. Products were analysed on polyacrylamide gels containing either 8% or 6% acrylamide. Reactions were primed using the ³²P-5'-labelled synthetic oligodeoxynucleotides¹⁷: 5AAAGCAGGGG14: 191TGC-TACTGAGCT202: 345CGCAGCAAAG354: 493GCAAAA-GGGG502: 623TCACCACCCG632: 777TGGACAATAG786: numbered according to the sequence of X-31 HA cDNA¹⁰. Those sequences shown from left to right are for viruses M1/HS8, M1/HS7 and M1/HS10 between nucleotides 738 and 780. The base changes are indicated by arrowheads and gel lanes contained from left to right ddGTP, ddATP, ddCTP and ddTTP terminated reactions. Nucleotide 742 which is a C is clearly separated from the two adjacent Cs only in the case of virus M1/HS10. Although this could indicate a C insertion in virus M1/HS10, this would alter the reading frame which would not be compatible with the fact that antigenically these viruses are indistinguishable and no other differences were detected between this variant and the wild type. For these reasons, coupled with the obvious differences in band intensity at this position in comparison with the other viruses, this region has been read as unchanged.

Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity

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The haemagglutinin (HA) glycoproteins of influenza virus membranes are responsible for binding viruses to cells by interacting with membrane receptor molecules which contain sialic acid (for review see ref. 1). This interaction is known to vary in detailed specificity for different influenza viruses (see, for example, refs 2-4) and we have attempted to identify the sialic acid binding site of the haemagglutinin by comparing the amino acid sequences of haemagglutinins with different binding specificities. We present here evidence that haemagglutinins which differ in recognizing either NeuAc α 2 $\rightarrow$ 3Gal- or NeuAc α 2 $\rightarrow$ 6Gal- linkages in glycoproteins also differ at amino acid 226 of HA1. This residue is located in a pocket on the distal tip of the molecule, an area previously proposed from considerations of the three-dimensional structure of the haemagglutinin to be involved in receptor binding⁵.

Comparison of receptor specificities of influenza viruses of the H3 subtype has revealed at least three distinct specificities based on preferential binding to either one or both of the sequences NeuAc α 2 $\rightarrow$ 6Gal α 1-4GlcNAc- or NeuAc α 2 $\rightarrow$ 3Gal α 1-3GalNAc- commonly found to terminate glycoprotein oligosaccharides linked to asparagine and to threonine or serine, respectively⁶. Preferential binding to the NeuAc α 2 $\rightarrow$ 6Gal-linkage was also found to correlate with high sensitivity to neutralization of infection by glycoproteins (γ inhibitors) present in horse serum⁷. This fact has allowed selection of receptor-specific variants from the recombinant virus X-31 (H3N2).

Variants were selected by growth of X-31 virus in hen eggs in the presence of non-immune horse or guinea pig sera. Table 1 compares X-31 and the selected variants for inhibitor sensitivity and for receptor specificity using human erythrocytes

Table 1 Receptor specificity and horse serum inhibitor sensitivity of variants of influenza virus H3 haemagglutinins

Virus*	Haemagglutination of erythrocytes†				Haemagglutination‡ inhibition HAI	Amino acid at residue 226§
	Native	Sialidase treated HA titre	NeuAcα2→3Gal	NeuAcα2→6Gal		
X-31	4,096	0	0	4,096	5,000	Leu
X-31/HS	4,096	0	4,096	0	30	Gln
X-31/GPS	512	0	256	0	30	Gln
M1/5	64	0	0	64	4,200	Leu
M1/HS10	64	0	0	64	8,100	Leu
M1/HS7	64	0	32	64	500	Met
M1/HS8	64	0	32	0	30	Gln

* Influenza virus X-31 (H3N2) was grown in hens' eggs in the absence (X-31) or presence of horse serum (X-31/HS) or guinea pig serum (X-31/GPS) and was purified as previously described¹⁶. Cloned variants of A/Memphis/102/72 (H3N2) were produced from a seed stock, obtained from Dr Robert G. Webster, by passage in MDCK cells in the absence (M1/5) or presence (M1/HS) of horse serum and subsequent plaque to plaque purification. HA was done either with MDCK cell lysates or with virus grown in hens' eggs. The titres are expressed as the reciprocal of the greatest dilution of virus at which agglutination occurred.

† Human erythrocytes were either unmodified (Native), treated with *Vibrio cholera* sialidase (sialidase treated) or sialidase treated and resialylated with CMP-NeuAc and purified sialyltransferases to contain the NeuAcα2→3Galβ1→3GalNAc or NeuAcα2→6Galβ1→4GlcNAc sequences as reported earlier⁴.

‡ Haemagglutinin inhibition (HAI) with horse serum was done by standard procedures with HAI titres expressed as the reciprocal of the greatest dilution that prevented agglutination by 4 HA units of virus.

§ Deduced from the nucleotide sequences of the HA1 region of the haemagglutinin genes. These were the only amino acid changes detected in HA1.

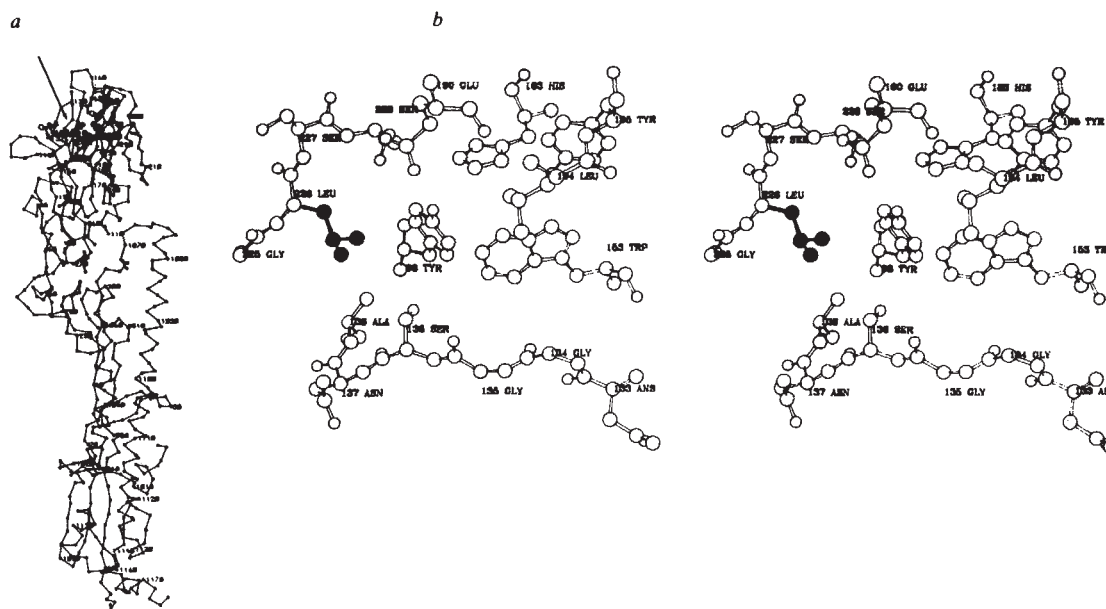


Fig. 2 *a*, Selected amino acid residues in and around the sialic acid receptor binding pocket are displayed on a schematic drawing of the α -C backbone of the haemagglutinin from the 1968 H3 influenza virus X-31. *b*, A detail of the same residues shown in *a* viewed from the top of the molecule. Leu 226 projects into the pocket near the central residue Tyr 98. The site illustrated recognizes NeuAcα2→6Gal- linkages while glutamine at position 226 alters the site to bind specifically NeuAcα2→3Gal- linkages.

specifically derivatized to contain the NeuAcα2→6Gal- and NeuAcα2→3Gal- linkages. While X-31 preferentially bound the NeuAcα2→6Gal- linkage and was very sensitive to inhibition of haemagglutination by horse serum, the selected variants bound the NeuAcα2→3Gal- linkage and were insensitive to haemagglutination inhibition.

In addition to these viruses, receptor-specific variants of A/Memphis/102/72 were obtained after growth of virus in MDCK cells in the presence and absence of horse serum and subsequent plaque purification. As indicated by the examples shown in Table 1, the viruses exhibiting preferential binding to the NeuAcα2→6Gal- linkage were inhibitor sensitive and those binding the NeuAcα2→3Gal- linkage were not. One variant which bound well to both linkages exhibited inhibitor sensitivity intermediate between the two extremes (Table 1).

The amino acid sequences of the haemagglutinins of these viruses were deduced from the nucleotide sequences of their

RNA genes. These were determined using dideoxynucleotide chain terminators⁸ in a primer extension system containing total virus RNA mixtures, reverse transcriptase and 5'-³²P-labelled synthetic oligodeoxynucleotide primers⁹. The complete sequences of the HA1 region of the haemagglutinin genes were determined for the seven viruses described in Table 1. The only amino acid sequence changes detected were at residue 226 as a result of base changes in the triplet nucleotides 754–756, CTG-leucine to CAG-glutamine or ATG-methionine (Fig. 1; see legend for discussion of an anomaly at nucleotide 742). As summarized in Table 1, amino acid 226 was leucine in viruses which preferentially bound the NeuAcα2→6Gal- linkage, glutamine for those that bound the NeuAcα2→3Gal- linkage and methionine for the variant that bound both linkages.

Examination of published amino acid sequences for the haemagglutinins of 22 influenza viruses of the H1, H2 and H3 subtypes indicates that leucine and glutamine are the only amino

acids reported for residues equivalent to 226 of haemagglutinins of the H3 subtype. This does not necessarily imply that, for example, glutamine at 226 will always specify NeuAc α 2 $\rightarrow$ 3Gal- recognition because other amino acid changes in the site may also alter its specificity. We have, however, analysed several antigenically different H3 viruses—for example, Aichi/2/68, A/Victoria/3/75, A/Texas/1/77 and A/Duck/Ukraine/63—and in each case the observed binding specificity corresponded to that predicted by the presence of leucine or glutamine at 226^{10–15} and the information presented in Table 1. Thus, despite a large background of amino acid variation observed in these haemagglutinins it is likely that the change at 226 accounts for the variations in their receptor specificity.

These observations on the modifications of sialic acid binding specificity as a consequence of amino acid substitutions at residue 226 directly support the tentative identification⁵ of the sialic acid binding site as a surface pocket at the distal end of the haemagglutinin molecule. This is illustrated in the α -carbon tracing of the X-31 haemagglutinin monomer shown in Fig. 2a. The proposal was originally based on the presence of conserved Tyr 98, His 183, Gln 190, Trp 153 and Leu 194, amino acid

residues in this pocket. Other conserved residues seem to stabilize the structure of the pocket without being in positions to interact with a receptor. Amino acid 226 is in this site (Fig. 2b), which is consistent with a direct role for this residue in receptor binding. We will not propose here a structurally detailed explanation for the differences in binding specificity as a result of the amino acid substitutions of glutamine and methionine for leucine because studies are in progress on crystals of haemagglutinins with different binding specificities and of haemagglutinin–sialyllactose complexes. Such studies of how single amino acid substitutions can change the specificity of haemagglutinin–sialic acid interactions may contribute to an understanding of the molecular bases of active site and receptor site binding specificities in general.

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Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta

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Cloned probes specific for unique genes have proven to be powerful tools in defining the nature of genetic diseases such as the thalassaemias¹ and growth hormone deficiencies². A similar approach should be useful in defining heritable diseases of type I collagen, the heterotrimer of two α 1(I) chains and one α 2(I) chain, which is the most abundant member of the collagen family of proteins. Recently, cloned cDNAs and genomic DNAs for the two polypeptide chains of the type I collagen^{3–10} have become available and have been used to elucidate the chromosomal location of the corresponding genes^{11–14}. Here, we have used several of these cloned DNAs to demonstrate the presence of an internal deletion of about 0.5 kilobases (kb) in one allele for the pro α 1(I) chain in a patient with osteogenesis imperfecta (OI), a group of heritable disorders which are characterized by brittle bones but which are highly heterogeneous both phenotypically and biochemically^{15,16}.

The patient had a severe form of OI which was lethal perinatally¹⁷. Cultured fibroblasts from the patient (ATCC, CRL 1262) were initially shown¹⁷ to secrete decreased amounts of type I procollagen, the large protein precursor of type I collagen. More recently, the patient's fibroblasts were shown

to synthesize equal amounts of normal-length and shortened pro α 1(I) chains^{18,19}. The shortened pro α 1(I) chains are incorporated into trimers of type I procollagen, but trimers containing the shortened pro α 1(I) chains have a decreased thermal stability and are rapidly degraded in fibroblast cultures¹⁹. The net effect is a marked reduction in the amount of type I procollagen available for fibre formation.

Total poly(A)⁺ RNA from CRL 1262 fibroblasts was translated in a rabbit reticulocyte cell-free system²⁰. The translation products contained normal-length and shortened pro α 1(I) chains in about equal amounts in addition to normal-length pro α 2(I) chains (Fig. 1). We then analysed the DNA of CRL 1262 fibroblasts by Southern blotting hybridization²¹. When a 1.8-kb cDNA covering the 3'-third of the gene⁹ (Hf-677 in Fig. 2) was used as a probe, the *Hind*III fragment detected was identical in size to that obtained with control DNA (Fig. 3a2). When a 1.8-kb cDNA covering the middle of the α 1(I) domain⁹ (Hf-404 in Fig. 2) was used as a probe, a shortened *Hind*III fragment of 8.5 kb was seen in addition to the normal fragments of 6 and 9 kb (Fig. 3a1). The results therefore suggested a deletion of 0.5 kb within the 9-kb *Hind*III fragment (Fig. 2). To explore this observation further, genomic DNAs from the CRL 1262 and control fibroblasts were digested with *Bam*HI and hybridized with three genomic subclones covering the same region (M.-L.C. *et al.*, in preparation). The 5'-most probe (2.2-kb *Bam*HI subclone in Fig. 2) showed no difference from the control (Fig. 3b3). The middle probe (2.0-kb *Bam*HI subclone in Fig. 2) revealed an additional 6.5-kb band in the DNA from the OI fibroblasts (Fig. 3b4). The additional 6.5-kb band was also seen with the 3'-most probe (2-kb *Bgl*II/*Hind*III subclone in Fig. 2) in the OI DNA (Fig. 3b5). The same 3'-probe hybridized to the short 8.5-kb band in *Hind*III digests of the OI DNA (Fig. 3c6). The data therefore indicated that one of the patient's alleles for pro α 1(I) contained a deletion of about 0.5 kb. The deletion eliminated the *Bam*HI site which is adjacent to an *Xho*I site found in both the gene and the cDNA and which includes the codon for amino acid residue 293 of the

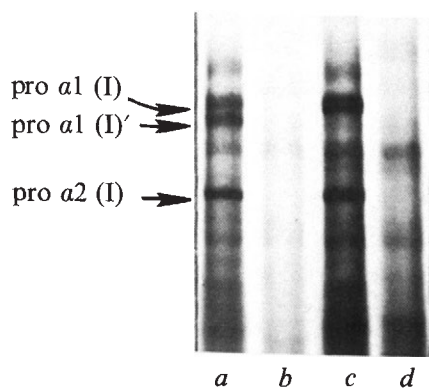


Fig. 1 Gel electrophoresis in SDS of cell-free translation products²⁰ of poly(A)⁺ RNA from OI fibroblasts (CRL 1262) and normal human fibroblasts (CRL 1106). About 0.15 µg of RNA was incubated in a final volume of 30 µl with ³⁵S-methionine. Lane a, OI (CRL 1262) translation products; lane b, same digested with bacterial collagenase²⁰; lane c, normal fibroblast (CRL 1106) translation products; lane d, same digested with bacterial collagenase.

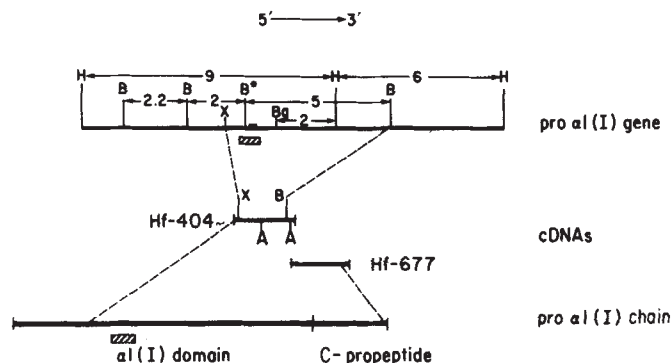


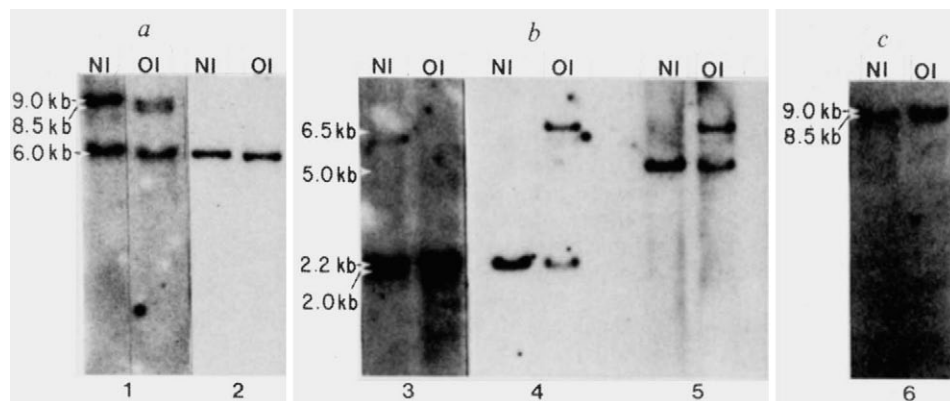
Fig. 2 Composite map of the pro α1(I) gene, two pro α1(I) cDNA clones and the pro α1(I) chain. The hatched boxes indicate the approximate location of the 0.5-kb deletion in the gene and the deletion of about 85 amino acids in the pro α1(I) chain. The solid box indicates the location of an *AluI* repetitive sequence. Numbers indicate lengths of DNA fragments in kb. Restriction endonuclease sites: H, *HindIII*; B, *BamHI*; Bg, *BglII*; X, *XhoI*; A, *AvaI*; B*, *BamHI* site missing from one allele. The *XhoI* recognition site is also cleaved by *AvaI*. The 5'-end of the cDNA clone Hf-404 codes for amino acid residue 247 of the α1(I) chain; the 3'-end of Hf-677 includes about 230 bp of the 3'-noncoding region of the mRNA. Both cDNAs are about 1.8 kb long.

α1(I) chain (Fig. 2). The deletion is also close to one of the *AluI* repeat sequences associated with the pro α1(I) gene (M.-L.C. *et al.*, in preparation).

To demonstrate that the gene deletion produced the shortening of the pro α1(I) chain, we examined RNA from the patient's fibroblasts in a series of S₁ nuclease protection experiments²². The DNA probe used in this analysis was a 675-base pair (bp) fragment of Hf-404 extending from the *XhoI* site to the adjacent *AvaI* site shown in Fig. 2 and coding for amino acid residues 293–518 of the α1(I) chain. When the DNA probe was ³²P-labelled at the 5'-end of the *AvaI* site and hybridized to poly(A)-enriched RNA from CRL 1262 fibroblasts before digestion with S₁ nuclease, a fragment of 320 bases was seen in addition to the fully protected fragment of 675 bases (left panel in Fig. 4). When the DNA probe was ³²P-labelled at the 3'-end of the *XhoI* site, a smaller fragment of 100 bases was seen (right panel

in Fig. 4). The contiguous *AvaI/AvaI* fragment from the 3'-side of the deletion was fully protected (not shown). The results indicated, therefore, that the genomic deletion of about 0.5 kb removed about 250 nucleotides which coded for sequences beginning at about amino acid residue 325 and extending to about amino acid residue 410 of the α1(I) chain (Fig. 2). This conclusion is consistent with data obtained by fragmenting the shortened pro α1(I) chain with vertebrate collagenase and which indicated that the shortening was within the first 775 amino acid residues¹⁹. Examination of the patient's fibroblasts also showed that the C-terminal propeptides of the shortened pro α1(I) chains associate normally with the C-terminal propeptides of normal-length pro α chains, and that the trimers containing the shortened pro α chains become triple-helical when cooled below 30 °C¹⁹. Therefore, the COOH-terminal half of the protein is functionally normal, and the deletion in the middle

Fig. 3 Southern blotting analysis of genomic DNA from OI fibroblasts (CRL 1262) and normal fibroblasts (CRL 1106). Hybridization with cDNA probes. *HindIII* digests with Hf-404 (panel a1) and Hf-677 (a2). b, Hybridization with three genomic DNA probes. *BamHI* digests of CRL 1262 DNA (OI) and CRL 1106 (N1) hybridized with the genomic subclones *BamHI* (2.2 kb) (b3); *BamHI/BamHI* (2 kb) (b4); and *BglII/HindIII* (2 kb) (b5). c, Hybridization with one genomic DNA probe. *HindIII* digests of CRL 1262 DNA (OI) and CRL 1106 DNA (N1) hybridized with the genomic subclone probe *BglII/HindIII* (2 kb) (c6). The numbers on the left side of the autoradiographs indicate the size of the fragments based on their migration relative to a *HindIII* digest of λ DNA.



Methods: Ten microgrammes of DNA from either source were digested to completion with the appropriate restriction enzyme, electrophoresed on a 0.7% agarose gel and blotted onto nitrocellulose filters²¹. Hybridizations were carried out at 40 °C for 25 h in 6×SSC (1×SSC: 0.15 M NaCl, 0.015 M Na citrate pH 6.8), 1× Denhardt's solution, 50 µg ml⁻¹ sheared salmon sperm DNA, 50% formamide and 5 ng ml⁻¹ of the appropriate probe nick-translated to a specific activity of 2–5×10⁸ c.p.m. per µg. At the end of the incubation the filters were washed, first at room temperature in 2×SSC, 0.1% SDS, then at 55 °C in sequentially decreasing concentrations down to 0.2×SSC, 0.1% SDS; then air dried and autoradiographed.

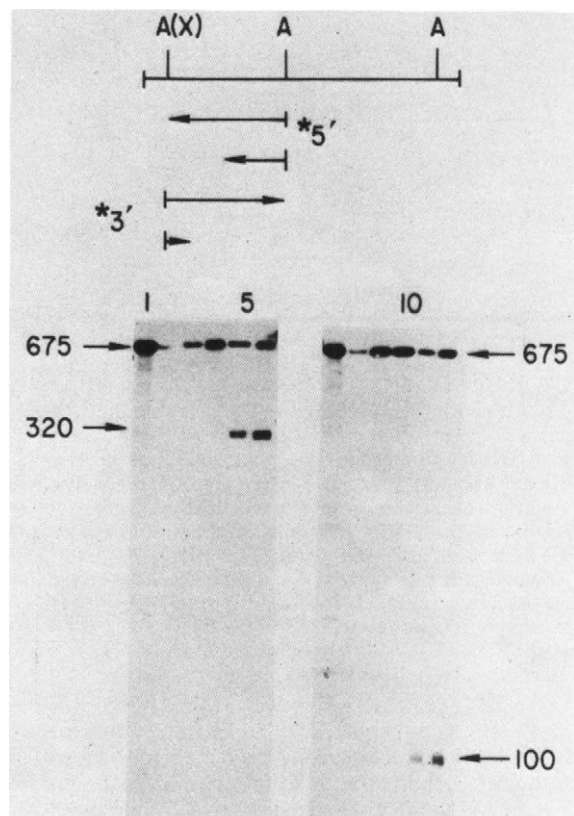


Fig. 4 S_1 nuclease protection experiments²² with the 675-bp cDNA ^{32}P -labelled at 5'-end of *Ava*I site (left panel) or 3'-end of *Xho*I (and *Ava*I) site (right panel). A map of the cDNA is presented in the top panel. Lane 1, control not digested with S_1 nuclease. Lane 2, control digested without any added RNA. Lane 3, protected with 0.5 μ g of control poly(A)⁺ RNA. Lane 4, protected with 4 μ g of control poly(A)⁺ RNA. Lane 5, protected with 1.0 μ g OI RNA. Lane 6, protected with 8 μ g OI RNA. Lanes 7–12, same with cDNA which was labelled at the 3'-end of the *Xho*I site.

Methods: The fragments obtained by digestion of Hf-404 with *Ava*I were labelled either at the 5'-end with [γ - ^{32}P]dATP and polynucleotide kinase or at the 3'-end with Klenow fragment of *Escherichia coli* polymerase and [α - ^{32}P]TTP²³ and then isolated by polyacrylamide gel electrophoresis. Total poly(A)⁺ RNA was hybridized with 1 ng of the double-stranded DNA fragment and digested with S_1 nuclease^{22,24}. The S_1 -nuclease-resistant fragments were run on a 5% polyacrylamide gel in 7 M urea. The gel was exposed for autoradiography with an intensifying screen at $-70^\circ C$.

of the gene apparently does not change the coding frame of the mRNA (Fig. 2).

Experiments are now in progress to define the exact location and extent of the deletion in the gene, the exons and introns involved and the role, if any, of the intronic *Alu*I repeat.

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Hepatoma secretory proteins migrate from rough endoplasmic reticulum to Golgi at characteristic rates

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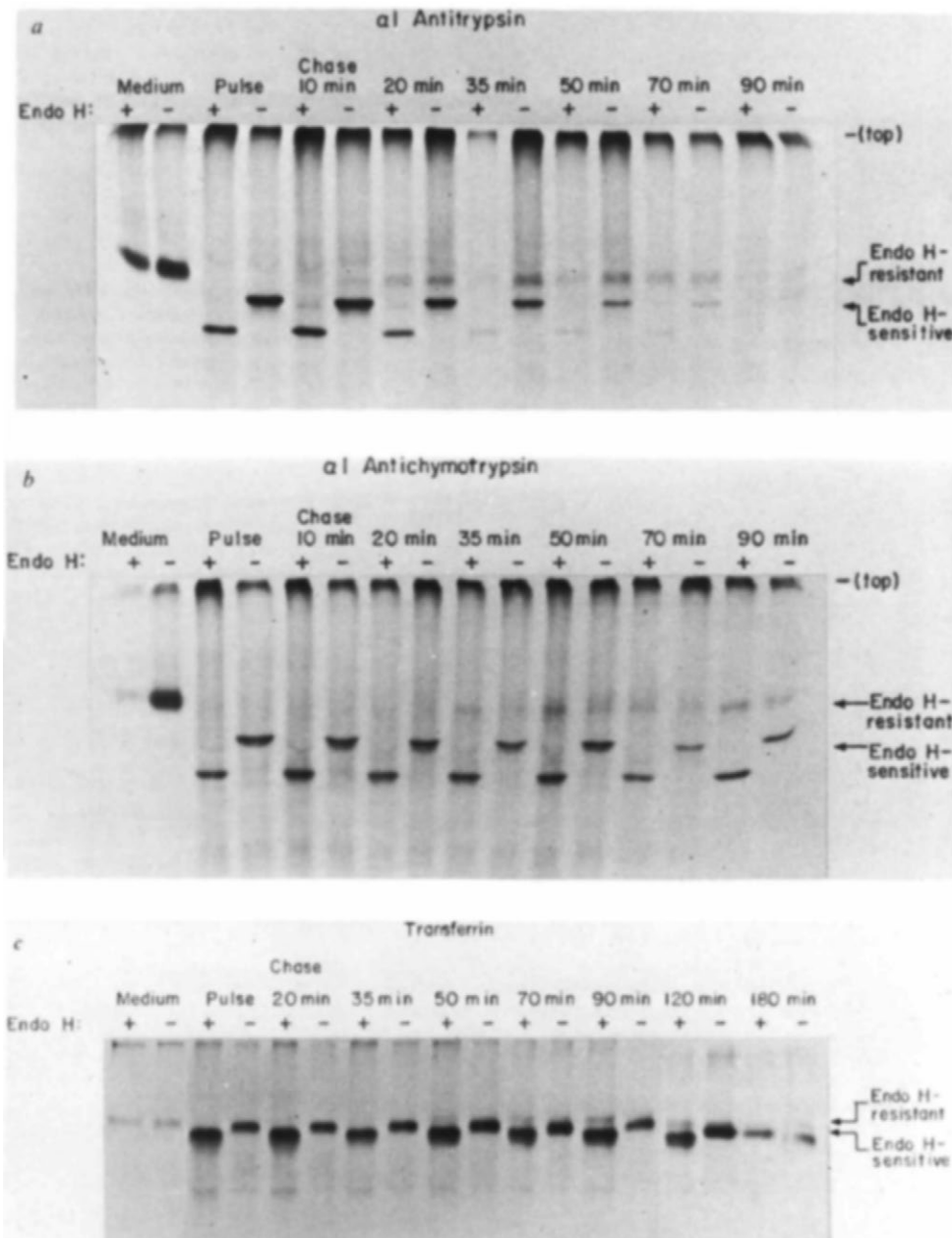
In eukaryotic cells, secretory proteins and glycoproteins migrate from the rough endoplasmic reticulum, their site of synthesis, through Golgi vesicles before being released from the cell^{1–9}. Cellular and viral integral plasma membrane glycoproteins are co-translationally inserted into the rough endoplasmic reticulum membrane and follow a similar pathway to the cell surface^{2,3,10–15}. Previous studies using endoglycosidase H (Endo H) suggested that in rat hepatoma cells the vesicular stomatitis virus (VSV) G protein, albumin and transferrin migrate from the rough endoplasmic reticulum to the Golgi apparatus at different rates¹⁶. Here we show directly that in human hepatoma HepG2 cells, five secreted proteins mature from the rough endoplasmic reticulum to Golgi vesicles at characteristic rates which differ at least threefold. The results are incompatible with bulk-phase movement of the luminal contents of the endoplasmic reticulum, and suggest that there is a membrane-bound receptor that selectively mediates the transport of secretory proteins from the rough endoplasmic reticulum to the Golgi.

HepG2 cells synthesize at least 20 serum proteins¹⁷. When HepG2 cells are labelled with ^{35}S -methionine at $32^\circ C$ for 10 min and then chased with unlabelled methionine, 50% of the labelled albumin and α_1 -antitrypsin is secreted in 45 min. Half of the labelled C3 complement and α_1 -antichymotrypsin are released in 85–90 min, whereas transferrin requires 140 min (data not shown, see Fig. 1 legend). C3 is synthesized as a precursor, pro-C3 (molecular weight (M_r) 187,000) which is largely cleaved intracellularly to two disulphide-linked subunits, α (M_r 115,000) and β (M_r 75,000)^{18,19}. About 20% of C3 is secreted as pro-C3, with the same kinetics as mature α and β C3 (not shown).

Endo H cuts the GlcNAc $\beta_1 \rightarrow 4$ GlcNAc bond in Asn-linked oligosaccharides, leaving only a single GlcNAc residue attached to the protein. Substrates for Endo H include the Glc₃Man₉GlcNAc₂ oligosaccharide transferred in the rough endoplasmic reticulum to Asn residues of glycoproteins, and processing intermediates such as Man₉GlcNAc₂, Man₈GlcNAc₂ and Man₅GlcNAc₂. Later intermediates in the processing to complex oligosaccharides²⁰ are resistant to Endo H^{21–23}. Figure 1a–c shows that pulse-labelled α_1 -antitrypsin, α_1 -antichymotrypsin and transferrin are all sensitive to Endo H, while the more slowly migrating mature forms secreted into the medium after a chase period are all resistant to Endo H. The level of intracellular pulse-labelled α_1 -antitrypsin that is resistant to Endo H reaches a steady-state value at 25 min of chase (at $32^\circ C$) and the level is maintained for 30–50 min (Fig. 1a). This material co-migrates with the secreted form. By contrast,

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Fig. 1 Digestion of ^{35}S -methionine-labelled proteins with Endo H. *a*, α_1 -Antitrypsin; *b*, α_1 -antichymotrypsin; *c*, transferrin. Culture dishes (60 mm diameter) containing about 3×10^6 growing HepG2 cells³⁰ were washed twice in complete phosphate-buffered saline (PBS), placed in 2.5 ml methionine-free growth medium (MEM medium, Gibco) containing 10% dialysed fetal calf serum (FCS) and incubated at 32 °C. After 20 min, 40 μCi ^{35}S -methionine (Amersham) was added, and the culture incubated for a further 10 min (pulse). The plates were washed twice with PBS, and 3 ml chase medium (growth medium plus 10% FCS and 1 mM methionine) was added to each. After incubation at 32 °C for the appropriate time (chase) the cultures were chilled on ice. The medium was saved and clarified by centrifugation at 10,000g for 10 min at 4 °C. The cells were washed three times in complete PBS, and lysed by addition of 1.4 ml lysis buffer (0.14 M NaCl, 0.004 M KCl, 0.001 M NaPO_4 ; 0.02 M Tris, pH 7.4; 1% sodium deoxycholate; 1% Nonidet P-40). The lysate was clarified by centrifugation for 10 min at top speed in an Eppendorf microcentrifuge, and used for quantitative immunoprecipitation. Medium from the sample after 180 min of chase was also used. Immunoprecipitations contained 300 μl lysis buffer, 100 μl lysate or 200 μl medium, and 10 μl of appropriate rabbit antiserum (Dako Immunoglobulins). After incubation at 4 °C for 1 h, with intermittent shaking, 50 μl of a 10% suspension of fixed, washed *Staphylococcus aureus* cells³¹ was added. Immunoprecipitates were recovered and washed as before³¹ except no SDS was used in the buffers. The immunoprecipitates were split into two portions; one was digested with Endo H (+) and the other mock-digested (-), as detailed previously³². Samples were analysed by SDS-gel electrophoresis and fluorography; a radioautogram of the dried gel is shown³². Arrows indicate the positions of the Endo H-resistant (or mature) form of the protein, and the Endo H-sensitive form. The fastest-migrating form of the protein, in the '+' lanes, is the species which had the high-mannose oligosaccharide removed. As there is an unavoidable loss of material during the multiple precipitations required for Endo H digestion, a direct analysis of a portion of the immunoprecipitates from the lysates and media by SDS-gel electrophoresis and fluorography was used to calculate the rate of secretion of the individual polypeptides (see text).



60 min is required for conversion of half of the pulse-labelled α_1 -antichymotrypsin to an Endo H-resistant form, and 120 min for transferrin. For all three proteins, the amount of labelled species in the intracellular Endo H-resistant pool, at steady state, is approximately that which is secreted in about 20 min. Thus, the differences in the secretion times of these proteins can be accounted for by differences in the time required for conversion of the oligosaccharides to Endo H resistance; only 20 min is required, on the average, for secretion of the mature proteins.

Processing of asparagine-linked oligosaccharides begins in the rough endoplasmic reticulum, where the three Glc residues (and possibly one Man residues)²⁴ are removed^{3,20}. The remainder of the processing events in the conversion to Endo H-resistant oligosaccharides occur in the Golgi apparatus. In particular, α -mannosidases Ia and Ib, the enzymes that convert $\text{Man}_5\text{GlcNAc}_2$ to $\text{Man}_5\text{GlcNAc}_2$, are Golgi enzymes²⁵⁻²⁷. Thus, it is important to determine whether the oligosaccharides in

the large intracellular pool of Endo H-sensitive secretory proteins is that characteristic of rough endoplasmic reticulum or Golgi processing intermediates. To this end, cells were labelled for 4 h with ^3H -mannose. Endo H-treated glycopeptides from intracellular transferrin were identified by gel filtration chromatography. Figure 2 shows that 57% of all oligosaccharides (71% of the Endo H-sensitive chains) have eight or more Man residues. $\text{Man}_5\text{GlcNAc}$ and $\text{Man}_3\text{GlcNAc}$, forms characteristic of the rough endoplasmic reticulum (M.S. and J.E. Rothman, unpublished), are the predominant species. No $\text{Man}_5\text{GlcNAc}$, a predominant Golgi processing intermediate, is evident. Thus, after a long labelling period, the vast majority of cell-associated transferrin is indeed in the rough endoplasmic reticulum; the lack of processing of its oligosaccharides would be due to the absence of migration of the protein to the Golgi apparatus.

To confirm that transferrin is transported to the Golgi at a much slower rate than is α_1 -antitrypsin or albumin, we carried

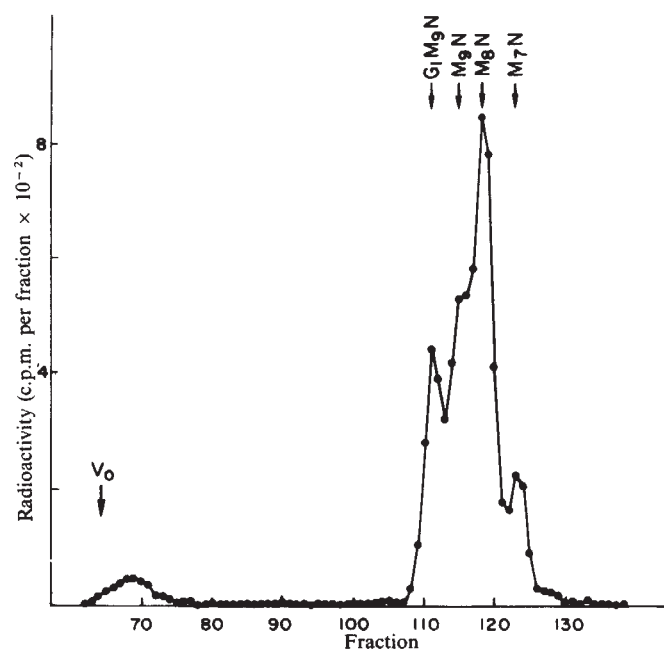
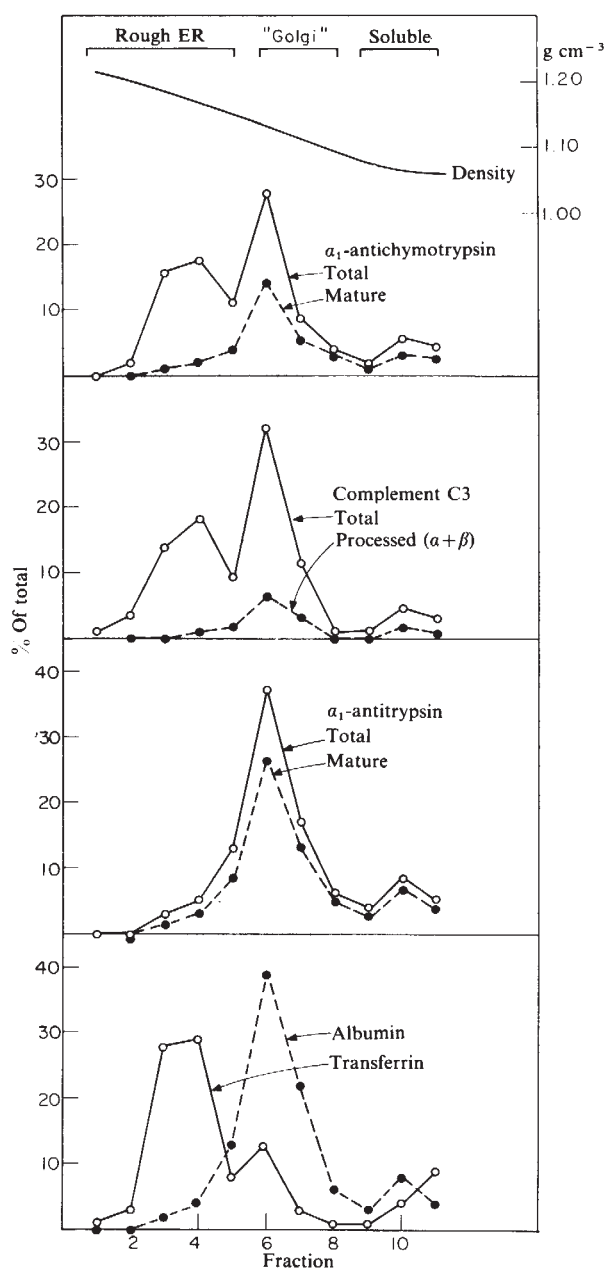


Fig. 3 Subcellular fractionation of the HepG2 cells pulse-chase-labelled with ^{35}S -methionine. Two 100-mm diameter culture dishes of HepG2 cells were labelled for 10 min with ^{35}S -methionine, then chased for 55 min at 32°C with unlabelled methionine. All subsequent procedures were done at $0-4^\circ\text{C}$. The cells were washed twice in complete PBS, scraped from the dish and resuspended in 2.5 ml buffer A (0.025 M HEPES, 0.05 M KCl, 0.002 M Mg acetate₂, pH 7.2, 1 mM dithiothreitol) containing 10% (w/v) sucrose. After swelling for 10 min, the cells were disrupted with 80 strokes in a tight-fitting Dounce homogenizer. Nuclei were removed by centrifugation at 1,000g for 10 min. 2.0 ml of cytoplasm was layered on 1.0 ml 65% (w/v) sucrose and 2.0 ml 15% sucrose, all in buffer A, in a tube designed for the SW50.1 Beckman ultracentrifuge rotor. Following centrifugation for 75 min at 40,000 r.p.m., the membranes were recovered with a syringe at the 15-65% sucrose interface. These were diluted to 15% sucrose with buffer A, and layered on a sucrose gradient (1.1 ml each of 65, 55, 50, 45, 40, 35, 30, 25 and 20% w/v, all in buffer A) in a tube designed for the SW41 Beckman ultracentrifuge rotor; this was centrifuged for 2 h at 40,000 r.p.m. Eleven equal 1.2-ml fractions were collected. Of each fraction, 100 μl were subjected to immunoprecipitation by each of the five rabbit anti-human serum proteins studied. For α_1 -antitrypsin, it was necessary to pre-clean the fractions first with normal rabbit serum and fixed *S. aureus* cells, followed by the immune serum and additional *S. aureus*. This was necessary to remove a minor nonspecific protein which migrated on the SDS gel near the Endo H-sensitive form of α_1 -antitrypsin. As in Fig. 1, the immunoprecipitates were analysed by SDS-gel electrophoresis and fluorography. The radioautogram was scanned and the areas under all peaks determined. For α_1 -antitrypsin and α_1 -antichymotrypsin, the radioactivity in the slowly migrating (Endo H-resistant) 'mature' form (dotted line) and the total radioactivity in both intracellular forms (solid line) is plotted as a percentage of the total radioactivity in this protein recovered from the gradient. Similarly, the radioactivity in the two processed forms of C3, α and β , and the total radioactivity in pro-C3, α and β , is plotted as a percentage of the total. The small amount of labelled protein at the top of the gradient represents material that has leaked from vesicles, and is not included in calculations summarized in the text.

Fig. 2 Analysis of ^3H -mannose oligosaccharides on intracellular transferrin. A 60-mm culture dish containing 3×10^6 HepG2 cells was placed in 2 ml low glucose medium (MEM containing only 0.1 mg ml^{-1} glucose and 10% dialysed FCS) and $200 \mu\text{Ci ml}^{-1}$ [^3H]mannose (17 Ci mmol^{-1} , NEN). After labelling for 4 h at 37°C , the cells were lysed in 1.4 ml lysis buffer. For immunoprecipitation, 0.9 ml of clarified lysate, 4.0 ml lysis buffer and 50 μl anti-transferrin antisera were incubated. The immunoprecipitate, recovered as for Fig. 1, was layered in a 5-cm slot on top of an SDS gel; ^{35}S -methionine-labelled transferrin was run in adjacent lanes as markers. The gel was fixed in 25% isopropanol-10% acetic acid, and dried from 10% acetic acid. The excised transferrin band, detected by autoradiography, was digested *in situ* with pronase as described previously³². The resulting glycopeptides were then desalted, digested with Endo H and analysed by gel filtration chromatography on BioGel P-4 (400-mesh) as previously described³². Arrows denote the void volume (V_0), determined with bovine serum albumin, and the migration positions of authentic Endo H-cleaved oligosaccharides: $\text{G}_1\text{M}_9\text{N}$, $\text{GlcMan}_9\text{GlcNAc}$; M_9N , $\text{Man}_9\text{GlcNAc}$; M_8N , $\text{Man}_8\text{GlcNAc}$; M_7N , $\text{Man}_7\text{GlcNAc}$. Each ^3H -oligosaccharide was also characterized by α -mannosidase digestion and by HPLC. When the labelled cells were incubated for 4 h in medium containing unlabelled mannose and glucose, all of the ^3H -labelled transferrin was secreted (not shown).



out subcellular fractionation experiments. Cells were either pulse-labelled with ^{35}S -methionine, or pulse-labelled and then chased for 55 min at 32 °C. A post-nuclear membrane fraction was prepared and centrifuged to equilibrium through a sucrose density gradient. We assume that labelled secretory protein which bands at 1.10–1.13 g cm^{-3} is in Golgi vesicles and not in some other smooth-surfaced vesicle, and that secretory proteins banding at 1.18 g cm^{-3} are in rough endoplasmic reticulum vesicles. Indeed, over 85–90% of pulse-labelled α_1 -antitrypsin, albumin, C3, α_1 -antichymotrypsin and transferrin bands in membranes at a density of 1.15–1.20 g cm^{-3} , the density of the rough endoplasmic reticulum (data not shown).

Importantly, after the 55-min chase period, 78% of labelled intracellular albumin bands at the density of smooth-surfaced membranes such as the Golgi. About 75% of the total vesicle-associated α_1 -antitrypsin and all of the mature Endo H-resistant α_1 -antitrypsin also bands at the density of the Golgi (Fig. 3). By contrast, only 20% of transferrin bands at this density; the remainder fractionates with the rough endoplasmic reticulum. Proteins such as C3 and α_1 -antichymotrypsin, which are secreted at an intermediate rate, are distributed on the density gradient in an intermediate fashion; about 50% are in the rough endoplasmic reticulum. All of the Endo H-resistant α_1 -antichymotrypsin, and all of the processed α and β C3 peptides, band with the Golgi, a result consistent with the notion that modification of oligosaccharides to the complex form and final proteolytic processing of C3 (and other proproteins) are events localized to Golgi or post-Golgi vesicles.

Clearly, the rate-limiting and distinctive step in intracellular maturation of these proteins is in movement from the rough endoplasmic reticulum to the Golgi. As judged from the rate at which proteins acquire resistance to Endo H (Fig. 1) and from the rate at which they are secreted, secretion of all proteins from the Golgi requires about the same period of time—20 min. In the exocrine pancreas all secretory proteins are found in all intracellular vesicles, suggesting that all proteins follow a common pathway^{7,14}. In VSV-infected rat hepatoma cells, albumin, transferrin and the VSV glycoprotein are also found in the same Golgi and pre-Golgi vesicles (G.J.A.M.S., unpublished observations), though in certain secretory cells there are two post-Golgi pathways for secretion¹⁵.

We do not know how proteins move from the rough endoplasmic reticulum to the Golgi. A common hypothesis is that small vesicles bud off from a specialized region of the rough endoplasmic reticulum, containing a sample of the luminal contents and rough endoplasmic reticulum proteins. These vesicles subsequently fuse with vesicles of the *cis*- (or proximal) region of the Golgi cisternae¹. Whether or not such vesicles contain a clathrin coat ('coated vesicles') is controversial^{13,25,28}.

We postulate that a receptor protein in the endoplasmic reticulum membrane regulates the selective transport of secretory proteins into transport vesicles *en route* to the Golgi. This receptor could be localized in the region of the rough endoplasmic reticulum from where the transport vesicles bud, and could bind most avidly those proteins which are transported to the Golgi at the faster rates. Slow-moving proteins, such as transferrin, need not bind at all to the receptor, and could be transported by bulk-phase movement of the luminal contents. By analogy to processes used for internalization of extracellular macromolecules, the transport of transferrin from the rough endoplasmic reticulum to the Golgi would resemble pinocytosis, while transport of albumin or α_1 -antitrypsin would resemble receptor-mediated endocytosis. Alternatively, a rough endoplasmic reticulum receptor could mediate the specific retention of secretory proteins in the rough endoplasmic reticulum.

After submission of this paper, Fitting and Kabat²⁹ showed that two viral glycoproteins mature from the rough endoplasmic reticulum to the cell surface at different rates, and suggested that transport of membrane proteins also requires a 'transport receptor'. The actual identification and characterization of such receptors remain to be performed.

Note added in proof: Ledford and Davis³³ have shown that albumin, α -fetoprotein and transferrin are secreted at different rates by a mouse hepatoma cell line.

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Left-handed helical conformation of poly[d(A-m⁵C) · d(G-T)]

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Poly[d(G-C)] serves as the prototype for the right-to-left (B to Z) transition in the helical sense of DNA, both in solution¹ and in the crystal form^{2,3}. However, the question remains as to which other synthetic and natural DNAs have the potential to adopt left-handed conformations. One logical candidate is the canonical alternating purine-pyrimidine sequence d(A-C)_n · d(G-T)_n, which seems to be widely disseminated in eukaryotic genomes^{4,5}. Our approach to this question is based on the enzymatic synthesis of poly[d(A-C) · d(G-U)] derivatives with systematic methyl and halogen substitutions in the C-5 position of the pyrimidines C and U. Such modifications in poly[d(G-C)] have previously been shown to potentiate the B to Z transition^{6,7}. Here we report a highly cooperative, reversible, salt- and temperature-dependent transition for poly[d(A-m⁵C) · d(G-T)], a repeat of the d(A-m⁵C) sequence which may occur in natural DNA⁸. Spectroscopic studies and the demonstrated ability to bind anti-Z-DNA antibodies suggest that the new helical conformation is left-handed and shares structural features with known Z-DNA. However, a novel property, not exhibited by poly[d(G-C)]¹, is the profound temperature dependence of the conformational equilibrium.

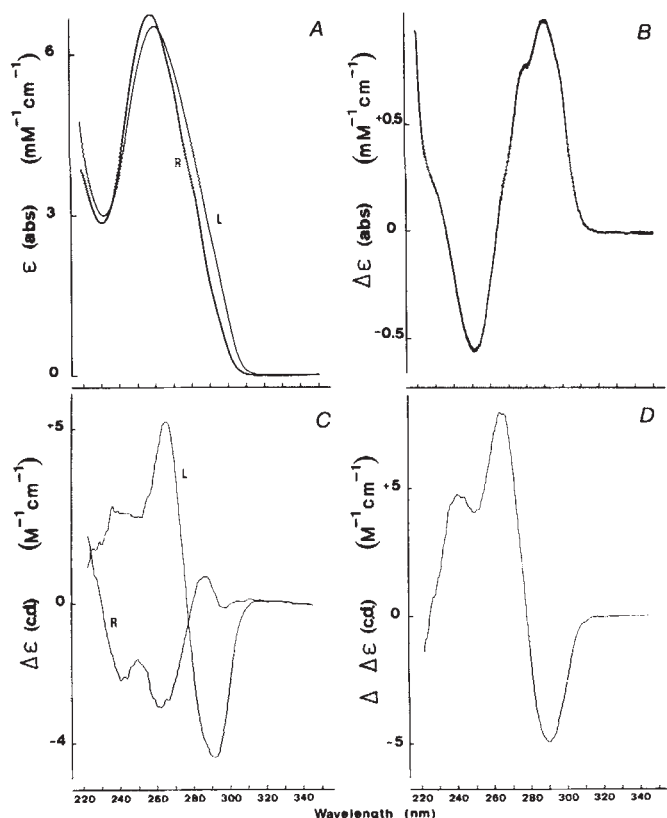


Fig. 1 Absorbance (A) and CD (C) spectra of poly[d(A-m⁵C)·d(G-T)] in 5.4 M NaCl, 11 mM Tris-HCl, pH 7.6, 0.1 mM EDTA at 46°C (R form) and 68°C (L form). As shown by the absorbance difference spectrum (B) (L-R), R-L transition is characterized by a red shift in the $\lambda_{\max}$ and by hyperchromicity at 290 nm. In addition, an inversion of the CD accompanies the transition, yielding the L-R difference spectrum shown in D. In low salt (10 mM NaCl) at 22°C, the absorption spectrum of poly[d(A-m⁵C)·d(G-T)] appears very similar to that shown in (A); the CD spectrum, however, is characterized by a larger positive $\Delta\epsilon$ and a shift in the negative intensity towards the 241 nm peak (data not shown). The polymer was synthesized using *Micrococcus luteus* DNA polymerase (PL Biochemicals) and dm⁵CTP (PL Biochemicals) as described elsewhere¹⁰. Nearest-neighbour analysis using [α -³²P]dATP and [α -³²P]dGTP showed >99% transfer to dm⁵CMP and TMP, respectively. The product had a T_m of 57.2°C in 1 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, and was 200–2,000 base pairs long as judged by gel electrophoresis. The molar absorptivity, $\epsilon_{260} = 6,700 \text{ M}^{-1} \text{ cm}^{-1}$, was determined by phosphate analysis. This value is slightly lower than that reported by Gill *et al.*⁴⁵. The UV absorption spectra were recorded with a Kontron Uvikon 820 and c.d. spectra were measured with a Jobin-Yvon Mark IV Dichrograph, calibrated with isoandrosterone. CD values are expressed as $\Delta\epsilon = \epsilon_L - \epsilon_R$.

Figure 1 shows the absolute and difference UV absorption and circular dichroism (CD) spectra of poly[d(A-m⁵C)·d(G-T)] determined in 5.4 M NaCl. Increasing the temperature from 46°C to 68°C results in a cooperative, reversible transition, characterized by a dramatic inversion of the CD spectrum (Fig. 1C, D) and a red shift in the UV absorption spectrum, with hypochromicity at 260 nm (Fig. 1A, B). These transitional properties are strikingly similar to those reported for the B → Z isomerization of poly[d(G-C)]^{1,9}. We designate the helical form in high salt and at high temperature as L (left) and the corresponding low-temperature and low- or high-salt B form as R (right). Figure 2 shows the reversibility and extreme cooperativity of the R-L transition elicited by small adjustments of either temperature or salt concentration. Variation of the ionic (NaCl) and thermal requirements for the R-L transition of this polymer demonstrates an inverse relationship between these two driving forces¹⁰; at 62.5°C, the midpoint of the R-L transition is 5.23 M NaCl, while in 5.4 M NaCl, the R-L thermal transition

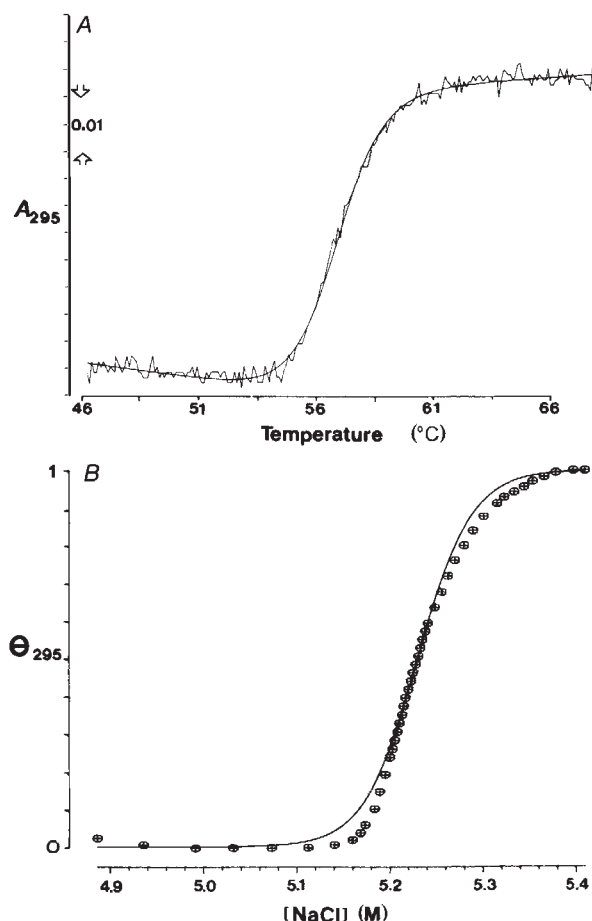
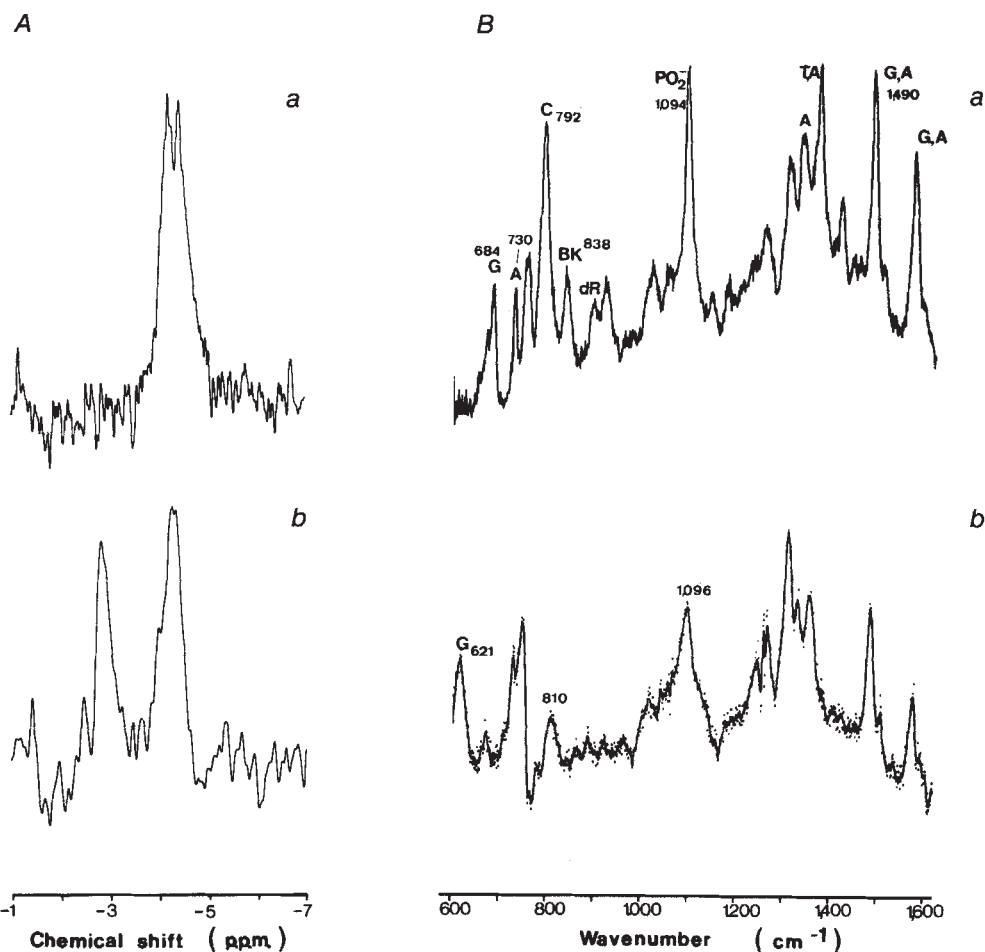


Fig. 2 The R-L transition of poly[d(A-m⁵C)·d(G-T)], monitored by the absorbance at 295 nm, depends on both temperature (A) and salt concentration (B). In 5.4 M NaCl, 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, an R-L isomerization occurs with heating (A). Application of curve fitting for a cooperative two-state transition, as described elsewhere⁴⁶ (smooth curve), yields a transition midpoint at 56.8°C, and an apparent $\Delta H = +0.92 \text{ MJ mol}^{-1}$ and a $\Delta S = +2.7 \text{ kJ mol}^{-1} \text{ deg}^{-1}$. The transitions were measured using a heating rate of $0.03^\circ \text{C min}^{-1}$ to ensure equilibration. Similarly, at a constant temperature of 62.5°C, a L-R transition of poly[d(A-m⁵C)·d(G-T)] may be observed by diluting the high-salt sample with 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA buffer. In 2B (points), the degree of transition, $\theta = [L]/([R] + [L])$, as determined from the measured sample absorbance at 295 nm, is plotted against NaCl concentration, after corrections for dilution effects and small linear absorbance changes in the pre- and post-transition regions. The titration midpoint was 5.23 M salt. Also shown is the theoretical curve for a co-operative two-state transition with this midpoint and a Hill coefficient of 180 (smooth curve); a similar value has been reported for the B-Z transition of poly[d(G-C)] in NaCl^{10,29}. The transition curve was obtained using a motor-driven syringe to inject 10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, at a rate of $0.406 \mu\text{l min}^{-1}$, into an absorbance cuvette containing a stirred 700- μl solution of DNA initially in saturated (5.4 M) NaCl.

midpoint is 56.8°C. Analysis of this thermal transition in the form of van't Hoff plot (not shown) yields an effective enthalpy change of $+0.92 \text{ MJ mol}^{-1}$ and an entropy increase of $+2.7 \text{ kJ K}^{-1} \text{ mol}^{-1}$. These results are in striking contrast to the temperature-independent ($\Delta H \sim 0$) B-Z transition of poly[d(G-C)] in NaCl¹ (although temperature-dependent kinetic effects are observed in the presence of divalent metal ions^{11,12}). At 62.0°C in 5.4 M NaCl, the R-L transition of poly[d(A-m⁵C)·d(G-T)] proceeds rapidly with a half time $t_{1/2}$ of 1.4 s. In MgCl₂ at 50.0°C, the midpoint for the L-R transition of poly[d(A-m⁵C)·d(G-T)] occurs at 1.8 M.

The B and Z conformations of DNA can be readily distinguished by ³¹P-NMR (ref. 13) and laser Raman^{14,15} analysis. Figure 3 gives the results of such measurements for poly[d(A-

Fig. 3 ^{31}P -NMR and laser Raman spectra of the R and L forms of poly[d(A- $m^5\text{C}$)·d(G-T)]. **A**, ^{31}P -NMR spectra of 2.9 mM poly[d(A- $m^5\text{C}$)·d(G-T)] in 30% (v/v) D_2O , 10 mM Tris-HCl, pH 7.6, 1 mM EDTA, and: **a**, 10 mM NaCl at 27 °C, or **b**, 5.4 M NaCl at 65 °C. The spectra were recorded using a Bruker WP200SY spectrometer, operating at 81.01 MHz, with ^1H -broadband decoupling. Chemical shifts are referenced to an external trimethyl phosphate sample, and negative values indicate an upfield shift relative to this standard. Line broadening of 5 and 6 Hz, respectively, was used. The low-salt R form resonances are at -4.25 and -4.48 p.p.m., whereas in high salt, the polymer resonances are at -2.84 and -4.32 p.p.m. **B**, Laser Raman spectra of: **a**, 40 mM poly[d(A- $m^5\text{C}$)·d(G-T)] in 220 mM NaCl, 4 mM Tris-HCl, pH 7.6, 0.4 mM EDTA at 25 °C (R form); and **b**, 31 mM poly[d(A- $m^5\text{C}$)·d(G-T)] in 5.4 M NaCl, 3 mM Tris-HCl, pH 7.6, 0.3 mM EDTA at 55 °C (L form). The spectra were recorded using the instrument of Dr M. Stockburger⁴⁷, using an excitation wavelength of 488 nm. The spectra are corrected for solvent contributions and in **b**, are smoothed (the unsmoothed data are shown as points). Some of the primary resonances which display changes accompanying the R-L transition are indicated (BK, backbone; dR, deoxyribose).



$m^5\text{C}$)·d(G-T)]. The ^{31}P -NMR spectrum of the DNA (Fig. 3A) at low salt concentration exhibits two closely spaced resonances separated by 0.23 p.p.m., a property shared with poly[d(A-T)] and currently interpreted in terms of an alternating B-DNA structure¹⁶⁻¹⁸. Indeed, many alternating purine-pyrimidine copolymers containing substituents at the C-5 positions of all pyrimidine bases display such ^{31}P -NMR peak splitting in low salt conditions^{10,17,19,20}. In 5.4 M NaCl and at 65 °C, the separation of these resonances increases dramatically to a value of 1.48 p.p.m. Such a ^{31}P -NMR spectrum is characteristic of the Z-DNA conformation^{9,10,13,18-21} and derives from the non-equivalent phosphate groups of the repeating dinucleotide structural unit⁹. By analogy to poly[d(G-C)] (ref. 9), we tentatively assign the downfield resonance to the purine-pyrimidine phosphodiester bonds.

Figure 3B shows the laser Raman spectra of poly[d(A- $m^5\text{C}$)·d(G-T)] in the R and L conformations (0.22 M NaCl at 25 °C and 5.4 M NaCl at 55 °C, respectively). Many of the features characteristic of the B-Z transition of poly[d(G-C)] (refs 14, 15, 22 and I.G. *et al.*, in preparation) are observed. Most striking is the shift in the guanosine ring breathing resonance from 684 cm^{-1} (R) to 621 cm^{-1} at high salt concentration and elevated temperature (L); such a shift is presumed to reflect the isomerization from an *anti* to a *syn* glycosidic bond configuration^{14,15}. The changed resonances associated with the backbone stretching vibrations (838 cm^{-1} and 792 cm^{-1} in the R form, 810 cm^{-1} in the L form) derive from alterations of the characteristic B-DNA C-2'-*endo* sugar pucker on passage to the left-handed conformation which, by analogy to Z-DNA, should exhibit some C-3'-*endo* character^{22,23}. Other changes in the PO_2^- symmetrical stretch and base ring stretch frequencies are observed, some of which are attributable to the bases A and T and thus reflect their disposition in the left-handed structure¹⁰.

Biochemical evidence for the left-handed nature of the L form of poly[d(A- $m^5\text{C}$)·d(G-T)] is provided by studies with antibodies specific for the structural determinants of Z-DNA. Other reports have documented recognition by antibodies of left-handed conformations of synthetic DNA^{7,24,25,32}, supercoiled DNA²⁵⁻³³, and condensed polytene chromosomes^{9,25,34-37}. We have compared the binding properties of a rabbit polyclonal anti-Z-DNA antibody (preparation T4 in refs 25 and 35) and a monoclonal antibody (D11, ref. 32; supplied by F. Pohl) tested on a radioactive poly[d(A- $m^5\text{C}$)·d(G-T)] probe. The former bound to the L (5.4 M NaCl, 63 °C) but not the R form to a level at least 50% of that displayed by a poly[d(G-C)] control. The monoclonal antibody, which has low affinity for poly[d(G- $m^5\text{C}$)]^{25,32}, demonstrated no recognition of poly[d(A- $m^5\text{C}$)·d(G-T)] in the L or R forms.

This report has been confined to the properties of poly[d(A- $m^5\text{C}$)·d(G-T)]; these are compared to those of poly[d(G-C)] in Table 1. Similar results have been obtained with modified poly[d(A-C)·d(G-U)] derivatives having different patterns of methyl and bromine substitution on the C-5 positions of the pyrimidine bases. The physical¹⁰ and immunochemical²⁵ properties of the entire family will be discussed elsewhere. Our findings are consistent with previous observations on poly[d(G-C)] in that the R-L transition is potentiated by substitutions at the C-5 position of the pyrimidine bases^{6,7}. However, the conditions in which the R-L transitions of this family occur are more stringent than those required for poly[d(G-C)] and its derivatives.

Poly[d(A-C)·d(G-T)] does not show an R-L transition in concentrated NaCl solutions on heating. However, recent electrophoretic³⁸ and immunochemical²⁸ evidence for a left-handed conformation of cloned d(A-C)_n·d(G-T)_n sequences under topological stress has been presented. Previous reports have attributed possible left-handed conformations to poly[d(A-C)·

Table 1 Comparison of polymer families displaying R-L transitions

Parameter	Poly[d(G-C)·d(G-C)]	Poly[d(A-m ⁵ C)·d(G-T)]
Spectroscopic properties		
UV absorbance (red shift)	+	+
Circular dichroism (inversion)	+	+
³¹ P-NMR (1.5 p.p.m. resonance splitting)	+	+
Raman (backbone and base resonance changes)	+	+
Transition properties		
Ease of transition	++	+
Effect of pyrimidine C-5 substitution on the transition	Enhanced (br > me)	Required
High co-operativity	+	+
Transition kinetics	+	+
Reversibility	+	+
Salt dependence (M ²⁺ > M ⁺ in effectiveness)	+	+
Temperature dependence	$\Delta H \sim 0$	$\Delta H \sim 1 \text{ MJ mol}^{-1}$
Structure		
Anti-Z-DNA antibody binding to L form	+	+
Associated L form (Z*, refs 9–12)	+	?
Crystal structure of L form	Z	?

The above properties apply to linear polymers.

d(G-T)] exposed to conditions required for fibre diffraction^{39,40}, to certain solvents and salts^{41,42}, and to acetylaminofluorene modification⁴³. However, these putative structures have yet to be confirmed by unambiguous spectroscopic studies. For example, in conditions such as concentrated CsCl in ethanolic solutions at low temperatures (<0°C), poly[d(A-C)·d(G-T)] displays large negative non-conservative CD spectra^{41,42}. We do not interpret these results as indicative of a left-handed conformation in view of the different spectroscopic and thermodynamic properties of the L form of poly[d(A-m⁵C)·d(G-T)] (Table 1). However, it can be anticipated that the poly[d(A-C)·d(G-T)] family of DNAs will assume a left-handed conformation *in vivo* in physiological conditions, that is, under the influence of proteins⁴⁴, topological strain^{28,38} and base modification (methylation)⁶. By extrapolation, we surmise that naturally occurring DNA consists of a hierarchy of sequences with respect to the potential for inter-conversion of helical sense.

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Inhibitor evidence for allosteric interaction in the replitase multienzyme complex

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We have previously shown that a fraction from the nuclei of S phase (DNA-synthesizing) Chinese hamster embryo fibroblasts (CHEF/18 cells) can be obtained that has a number of the enzyme activities required for DNA biosynthesis, and can catalyse the incorporation of labelled precursors into DNA (refs 1–4, also see ref. 8). This fraction, which we have termed the 'replitase', contains spherical particles of diameter ~25 nm, apparently multienzyme complexes for *de novo* DNA biosynthesis. Here we present evidence for the functional association of one of the enzyme activities, thymidylate synthase, with several of the other enzyme activities. Hydroxyurea, novobiocin and aphidicolin, inhibitors of ribonucleotide reductase, topoisomerase and DNA polymerase α , respectively, all inhibit thymidylate synthase in intact S phase CHEF/18 cells, but not in their soluble extracts. We suggest that these results reflect allosteric interactions between the subunits of a multienzyme DNA-synthesizing complex, which can be modulated by the specific inhibitors of individual enzyme activities in intact cells.

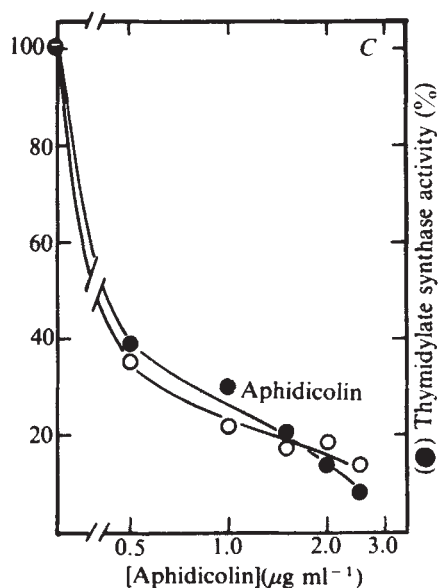


Fig. 1 Effect of hydroxyurea (x), novobiocin (Δ) and aphidicolin (■) on thymidylate synthase activity in soluble extracts of S phase CHEF/18 cells. ●, Control. CHEF/18 cells were grown and synchronized by isoleucine starvation as described elsewhere². When the cells were in mid-S phase, 12 h after release from an isoleucine block, they were trypsinized and suspended in ice-cold Dulbecco's modified Eagle's medium (DMEM). These cells were pelleted and resuspended in buffer A (mM): 150 sucrose, 80 KCl, 35 HEPES pH 7.4, 5 potassium phosphate pH 7.4, 5 MgCl₂, 0.5 CaCl₂, at a density of 5×10^7 cells ml⁻¹ and homogenized in a Potter-Elvehjem glass homogenizer by applying 10 strokes at setting 3 in a Sorvall Omni-mixer (type 17105, DuPont Instruments) with a teflon pestle. The nuclei were obtained by centrifugation at 850g for 10 min. The nuclear pellet was suspended in ice-cold buffer A to a density of $\sim 5 \times 10^7$ nuclear particles ml⁻¹, then sonicated in four pulses of 30 s each, using a Branson Sonifier (Model 185) at setting 2. The nuclear sonicate was centrifuged at 25,000 r.p.m. at 4 °C for 1 h in a Beckman SW 50.1 rotor with an adapter which allows the use of 0.8-ml tubes, and the supernatant was used as the soluble extract for thymidylate synthase assays, measured as described elsewhere¹. Inhibitors were included in the reaction mixture at the following concentrations: hydroxyurea, 0.5 mM; novobiocin, 0.5 mM; aphidicolin, 10 μg ml⁻¹.

As shown in Fig. 1, hydroxyurea, novobiocin and aphidicolin had no inhibitory effects on thymidylate synthase activity in crude soluble extracts of S phase CHEF/18 cells as measured by the ³H-H₂O formed from added [5-³H]dUMP on its conversion to dTMP^{5,6}. Thymidylate synthase, *per se*, is not sensitive to these inhibitors. In striking contrast, as shown in Fig. 2A-C, at similar or lower concentrations, they caused dramatic parallel inhibitions of thymidylate synthase activity and DNA synthesis measured in intact cells. These results demonstrate that thymidylate synthase activity is inhibited in intact cells by three agents which affect three other enzymes associated with DNA biosynthesis.

These observations could be accounted for if a metabolite such as dTTP accumulated during inhibition of DNA synthesis,

and if this metabolite feedback-inhibited thymidylate synthase, or affected transport of ³H-dUrd into the cell or kinase activity. The first condition seems unlikely because hydroxyurea prevents accumulation of deoxynucleotides⁷. The second condition appears to be ruled out because thymidylate synthase activity in permeabilized cells was not inhibited by excess dTMP or dTTP (50 μM) when the substrate, dUMP, was only 16 μM (ref. 1). Furthermore, hydroxyurea did not limit the accumulation of radioactive thymidine in acid-soluble pools of L929 cells⁷.

Thymidylate synthase activity is present throughout the cell cycle, but in intact or permeabilized cells it is high only when the cells are in S phase^{1,5}. During this phase thymidylate synthase is part of a sedimentable entity¹. From these observations

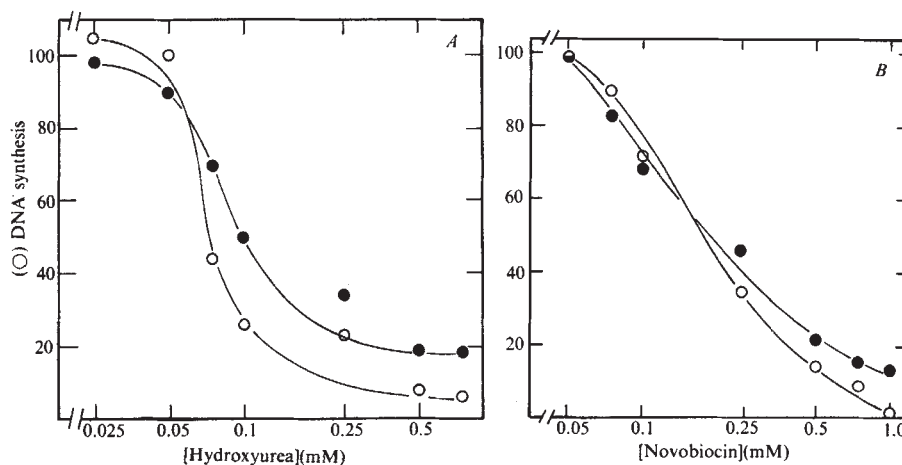


Fig. 2 Effect of inhibitors on DNA biosynthesis and thymidylate synthase activity in intact S phase CHEF/18 cells. A, Effect of hydroxyurea; B, effect of novobiocin; C, effect of aphidicolin. Open symbols, [6-³H]dUrd incorporation; solid symbols, ³H-H₂O formed from [5-³H]dUrd. **Methods:** CHEF/18 cells were synchronized as described in Fig. 1 legend. Duplicate culture dishes were pulsed for 1 h at 37 °C with undiluted 5 μCi ml⁻¹ [6-³H]dUrd (24.5 Ci mmol⁻¹; NEN) or with 10 μCi ml⁻¹ [5-³H]dUrd (13 Ci mmol⁻¹; Schwarz Mann). Cells pulsed with [6-³H]dUrd were processed to estimate the rate of DNA biosynthesis by washing monolayers twice with ice-cold phosphate-buffered saline (PBS), three times with 10% (w/v) trichloroacetic acid (TCA) and extracting the acid-insoluble material with 1 ml NaOH (0.2 M). Aliquots (100 μl) of alkaline extract were counted to determine moles of deoxyuridine incorporated into DNA. In control culture dishes, 100% [6-³H]dUrd incorporation into acid-insoluble material was equivalent to 6.9 ± 0.2 pmol per 10⁶ cells per min. Culture dishes pulsed with [5-³H]dUrd were processed as follows to estimate thymidylate synthase activity in intact cells: the medium from these dishes was collected without disturbing the monolayer and analysed for ³H-H₂O content formed by synthesis of dTMP from [5-³H]dUMP, within the cell⁵. ³H-H₂O was separated from [5-³H]dUrd by filtering the medium through a 1-cm charcoal (activated Norit) bed in a Pasteur pipette, which retained >99% of the [5-³H]dUrd. The filtrate contained all the released tritium as ³H-H₂O; 100 μl of filtrate were counted for radioactivity. In control cultures 100% ³H-H₂O formed from [5-³H]dUrd was calculated to be 6.0 ± 0.5 pmol per 10⁶ cells per min. To test the effect of inhibitors, various concentrations of aphidicolin (Wako), hydroxyurea (Sigma) or novobiocin (Sigma) were added to the culture dishes 30 min before pulsing with radioactive nucleosides, and processed as described above.

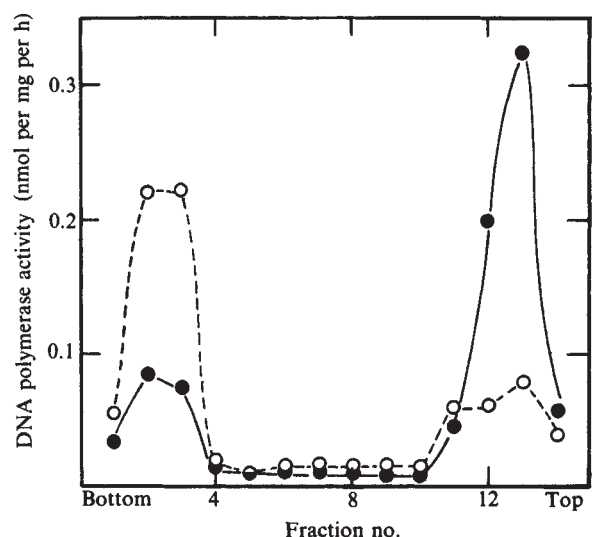


Fig. 3 Release by novobiocin of association of DNA polymerase with the multienzyme complex for DNA replication (replisome). ○, DNA polymerase activity in fractions obtained from the control nuclear lysate gradient; ●, DNA polymerase activity in fractions obtained from the novobiocin-treated nuclear lysate gradient. Cells were synchronized to G_0/G_1 phase of the cell cycle by isoleucine starvation as described elsewhere²; 12 h after releasing from isoleucine block, when the cells were in mid-S phase, 0.25 M novobiocin dissolved in PBS was added to the culture medium to a final concentration of 0.25 mM. Control cultures received equal volumes of PBS. After 1 h of treatment, cells were trypsinized, suspended in ice-cold medium (DMEM), pelleted and resuspended in buffer A with 8 mM dithiothreitol (DTT) at a density of 1×10^8 cells ml^{-1} . This cell suspension was homogenized and the nuclei were recovered as a pellet as described in Fig. 1 legend. The nuclear pellet was suspended in the same volume of buffer A with 8 mM DTT, then sonicated gently with 2 pulses of 20 s each using a Branson Sonifier at setting 1. This nuclear sonicate was clarified by centrifuging at 3,000 r.p.m. for 10 min, then 0.4 ml of the supernatant was layered onto a linear gradient of 10–40% (w/w) sucrose in buffer A containing 8 mM DTT, which was overlaid on 66% (w/w) sucrose (0.4 ml). These sucrose density gradients were centrifuged in a Beckman SW 50.1 rotor at 4 °C for 8 h at 30,000 r.p.m. and were resolved into 0.4-ml fractions. DNA polymerase activity in individual fractions was measured as described elsewhere².

we suggest as a working hypothesis that if an inhibitor conformationally alters its target enzyme, it may positively or negatively alter allosteric interactions between this and other enzymes of the replisome. In the search for such changed conformational interactions, we found that treatment of intact S phase cells with novobiocin made the DNA polymerase activity move from a sedimentable nuclear fraction to a low-molecular weight fraction (Fig. 3). In addition, after novobiocin treatment, only a small portion of total thymidylate synthase activity (86 pmol per mg per h) was recovered in the nuclear fraction compared with the control (324 pmol per mg per h). The two other inhibitors did not measurably alter the sedimentation characteristics of the enzyme. All the inhibitors of enzymes in replisome do not necessarily cause marked conformational changes; or, if they do, the consequences could be allosterically positive, negative or neutral for other affected enzymes (for example, positive effect of hydroxyurea on DNA polymerase²).

Further studies will be necessary to test how other modifiers of the DNA biosynthetic pathway affect thymidylate synthase and the other activities in intact normal cells compared with transformed cells. Such studies are important, not only to understand allosteric interactions between the enzymes involved in DNA biosynthesis, but they may also be valuable in identifying new target sites for chemotherapeutic attack or in designing

improved strategies for the use of existing agents in cancer treatment.

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Note added in proof: After submitting this manuscript, nucleotide pool analysis data indicated that none of these antimetabolites (hydroxyurea, aphidicolin or novobiocin) dramatically influenced the uptake of dUrd and its subsequent phosphorylation to dUMP. These antimetabolites also did not allow greater accumulation of dTMP, dTDP or dTTP inside the cell compared to the control.

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***In situ* nick-translation distinguishes between active and inactive X chromosomes**

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Template-active regions of chromatin are structurally distinct from nontranscribing segments of the genome. Recently, it was suggested that the conformation of active genes which renders them sensitive to DNase I may be maintained even in fixed mitotic chromosomes. We have developed a technique of mitotic cell fixation and DNase I-directed nick-translation which distinguishes between active and inactive X chromosomes. We report here that *Gerbillus gerbillus* (rodent) female cells contain easily identified composite X chromosomes each of which includes the original X chromosome flanked by two characteristic autosomal segments. After nick-translation the active X chromosome in each cell is labelled specifically in both the autosomal and X-chromosomal regions. The inactive X chromosome is labelled only in the autosomal regions and in a small early replicating band within the late replicating 'original X' chromosome. Our technique opens the possibility of following the kinetics of X-chromosome inactivation and reactivation during embryogenesis, studying active genes in the inactive X chromosome and mapping tissue-specific gene clusters.

A considerable amount of data indicate that template-active regions of chromatin are structurally distinct from nontranscribing segments of the genome. The enzymes DNase I, DNase II and micrococcal nuclease preferentially nick active chromosomal segments and thus serve as structural probes for gene expression^{1–5}. DNase I sensitivity which results from the specific conformation of active genes is in many cases independent of the degree of gene transcription⁶. Thus, the adult β -globin gene in chick embryos is already sensitive to DNase I in the red cell precursors during erythropoiesis before it is expressed⁷ and is also sensitive in chick erythrocytes which have ceased to synthesize β -globin¹. All these data are consistent with the notion

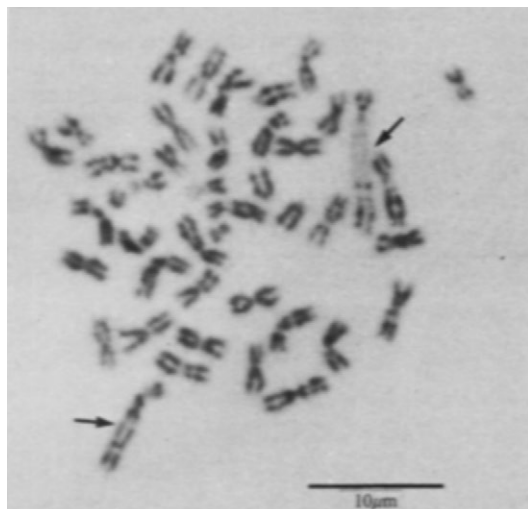


Fig. 1 Typical mitotic spread of *G. gerbillus* lung primary fibroblast cell labelled during late S phase with 5-bromo-2-deoxyuridine (5-BrdUrd) and stained by the combined 33258-Hoechst-Giemsa method¹¹ to reveal the 5-BrdUrd-containing chromosomal regions (replication bands). The upper arrow indicates the late replicating inactive X chromosome, and the lower arrow the early replicating active X chromosome. The early replicating and therefore darkly stained chromosomal regions in the inactive X chromosome include two autosomal regions—AI and AII—which flank the original X chromosome and a small band within it⁹. Most of the chromosomal regions of the active X chromosome replicated early, did not incorporate 5-BrdUrd and are therefore darkly stained both along the autosomal segments and the X-chromosomal regions. The cells were grown at 37°C in 5% CO₂ in Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum, penicillin (100 μg ml⁻¹) and streptomycin (100 μg ml⁻¹). 5-BrdUrd (final concentration 10⁻⁵ M) was added to the cell culture 4 h before collection and incubation was continued in the dark. Colcemid (final concentration 0.05 μg ml⁻¹) was added to arrest cells at metaphase 30 min before collection. Cells were collected and treated with hypotonic KCl (0.5%) solution for 10 min at 37°C, fixed with methanol/acetic acid (3:1) for 1 h, dripped on microscope slides and air-dried.

that DNase I sensitivity of genes is correlated with their potential activity and is retained even when the genes are temporarily silent. Recently, it was shown that active genes are sensitive to DNase I to the same degree in mitotic chromosomes and interphase nuclei⁸, suggesting that active regions might be visualized by autoradiography following *in situ* nick-translation of metaphase chromosomes. The specificity of this technique was indicated by the fact that over 70% of the silver grains in such autoradiographs were symmetrically placed on sister chromatids⁸.

We have further studied the DNase I sensitivity of DNA in fixed mitotic chromosomes in order to determine whether active genes retain their high differential sensitivity to this enzyme and hence can be mapped *in situ*. We studied the differential sensitivity to DNase I of DNA in active and inactive X chromosomes. *G. gerbillus* lung primary fibroblast cells have very long, easily identifiable X chromosomes which are composed of 'original X' material flanked by autosomal stretches⁹. The autosomal regions and a small band within the 'original X' chromosome replicate in the inactive X chromosome much earlier than the 'original X' chromosomal regions and thus can be identified as dark replication bands in metaphases labelled with 5-bromodeoxyuridine (5-BrdUrd) during late S phase⁹ (Fig. 1). The autosomal regions in the active X chromosome (AI and AII) also replicate early (Fig. 1). In cells in which the inactive X chromosome incorporated 5-BrdU, this chromosome was sometimes longer than the active X chromosome as the incorporated 5-BrdUrd interferes with chromosome condensation¹⁰. However, as previously observed^{11,12} in unlabelled metaphase spreads, the inactive X chromosome has a different morphological appearance and is usually shorter than the active X, allowing these two chromosomes to be distinguished in the absence of 5-BrdUrd treatment.

Figure 2A shows a typical mitotic cell nick-translated *in situ*. In this preparation it can be observed that one X chromosome is labelled, while the second X chromosome is practically free of label in its X-chromosomal region (OX). All the autosomes are labelled. The label is usually placed symmetrically on sister chromatids which exhibit labelled and unlabelled segments.

In this study 143 nick-translated mitotic cells were screened. In 26 of the cells, the labelling efficiency of the chromosomes was very low (<20 grains per cell) and these cells could not be

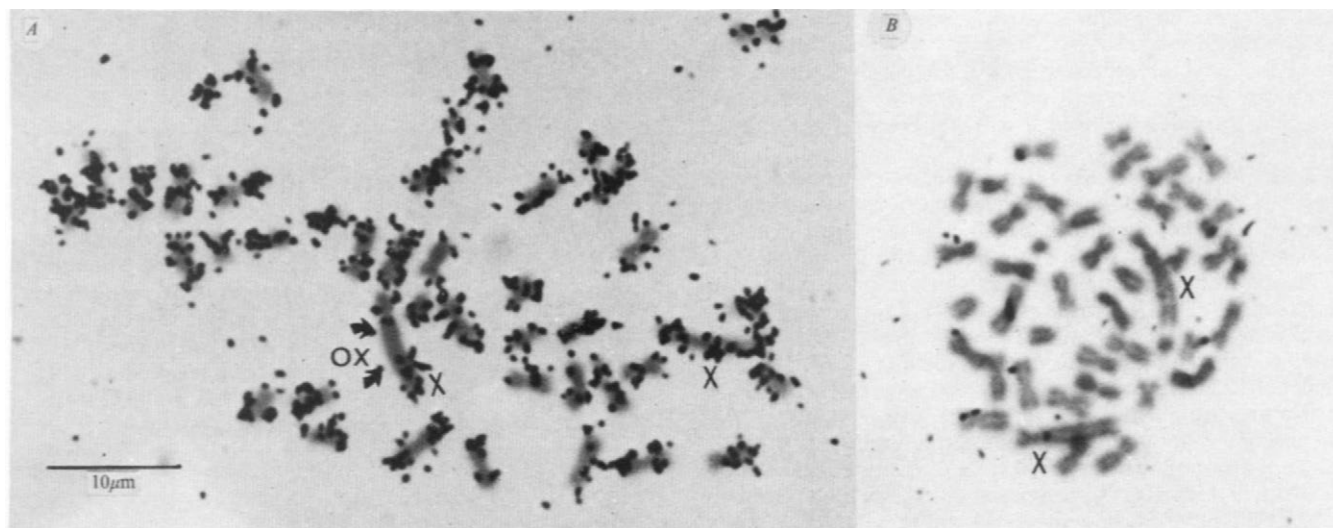


Fig. 2 Autoradiography of typical nick-translated metaphase spreads of female *G. gerbillus* primary lung fibroblasts. A Represents *in situ* nick-translations using DNase I; B shows the results obtained when the reaction was carried out in the absence of DNase I. The two X chromosomes (X) and the 'original X' region (OX) are marked. These cells were grown and the mitotic spreads prepared as described in Fig. 1 legend. Immediately after fixation the slides were incubated for 5 min at room temperature in 100 μl nick-translation mixture which contained: 50 mM Tris-HCl pH 7.9, 5 mM MgCl₂, 10 mM 2-β-mercaptoethanol, 50 μg ml⁻¹ bovine serum albumin, DNA polymerase I (10 U ml⁻¹; Boehringer Mannheim), pancreatic DNase I (20 ng ml⁻¹), 4 μM dATP, dCTP and dGTP, and 0.3 μM ³H-dTTP (40–50 Ci mmol⁻¹; NEN). The reaction was terminated by washing the slides in running tap water. The slides were then rinsed in ethanol and incubated for 5 min in 5% TCA at 4°C. The dried slides were covered with photographic emulsion (Kodak NTB2) and stored in the dark at 4°C for 1 month. The autoradiographs were developed in D-19 (Kodak) and the mitotic preparations were stained with 2% Giemsa.

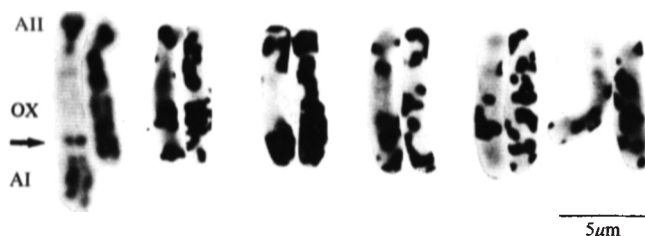


Fig. 3 Six pairs of X chromosomes from metaphase spreads of female *G. gerbillus* primary lung fibroblasts. In each pair the left chromosome is the inactive X chromosome. The first pair (on the left), which show replication bands following 5-BrdUrd treatment, was taken from Fig. 1. The autosomal regions, AI and AII, and the 'original X' chromosome (OX) are marked. The arrow indicates the early replicating band within the original X chromosome. The other five pairs of X chromosomes illustrate representative autoradiographs of nick-translated mitotic cells; in each pair the inactive X chromosome is free of label except for the labelled early replicating segments which include the autosomal regions AI and AII and the small band within the OX. In some of the inactive X chromosomes which have been underlabelled, one or even two of these regions are hardly labelled. However, the inactive X-chromosomal regions are always free of label. The active X chromosome—the right one in each pair—is labelled along both the autosomal stretches and the X-chromosomal segments.

used to identify the activity state of the X chromosomes. Of the 117 labelled metaphase plates, 93 mitotic cells (79%) show differential labelling of the two X chromosomes. In all cases, the active X chromosome was labelled both in the autosomal (AI and AII) and X-chromosomal regions while the inactive X chromosome was labelled in the autosomal regions and in the early replicating band within the 'original X' chromosome. As the inactive X appeared slightly shorter in most of these preparations it was possible to show that the unlabelled chromosome corresponded to the inactive X. Examples of *in situ* nick-translated X chromosome pairs from several individual cell preparations are shown in Fig. 3.

In 24 nick-translated mitotic cells (21%) we observed very heavy labelling and in these cells both X chromosomes were found to be labelled nondifferentially. It is possible that some cells are affected by the fixation procedure so as to cause nonspecific DNase I nicking. Note in this regard that all labelling observed in these experiments was DNase I-dependent. Very few grains (10–15 per cell) were observed in all the screened preparations that were not treated with DNase I (see Fig. 2B and ref. 8).

These results show that in fixed mitotic chromosomes the active X chromosome is more sensitive to DNase I than the inactive X. Whereas most chromosomes contained extensive regions of DNase I sensitivity, the inactive X chromosome appeared to be in a conformation which made it resistant to this enzyme. This lack of sensitivity was in apparently sharp contrast to two internal positive controls, the active X chromosome and the autosomal segments of the inactive X. Thus, the DNase I-sensitive conformation typical of many active genes is also present in active X chromosome genes but absent from the inactive genes in the inactive X chromosome.

It is interesting that the small early replicating region within the "original inactive X chromosome"⁹ shows high sensitivity to DNase I indicating its active DNA conformation. Previously, it was shown that human female inactive X chromosomes include some genes which escape inactivation^{13,14}. One such gene, the steroid sulphatase (STS) gene, has been mapped to an early replicating region of the inactive X chromosome^{15,16}. These results and others led to the assumption that early replicating regions in the inactive X chromosome remain active¹⁷. The use of our technique allows identification of active regions in the inactive X chromosome and the study of any correlation between early replicating regions in this chromosome and gene

activity. This technique should be useful for following the kinetics of X-chromosome inactivation and reactivation in individual cells during animal embryogenesis and germ cell formation. Furthermore, it may allow the identification and mapping of active gene clusters including tissue-specific genes.

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Corrigendum

In the letter 'Direct surface imaging in small metal particles' by L. D. Marks and David J. Smith, *Nature* **303**, 316–317 (1983), in the second sentence of the third paragraph on page 317, the value $\langle 1\bar{1}1 \rangle$ should read $\langle 1\bar{1}0 \rangle$.

Erratum

In the letter 'Characterization of rat hypothalamic growth hormone-releasing factor' by J. Spiess, J. Rivier and W. Vale, *Nature* **303**, 532–535 (1983), in the final sentence of the third paragraph on page 533, 'Ca²⁺-independent' should be 'Ca²⁺-dependent'.

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Environmental lobbies: consensus or confrontation?

Eric Ashby

Environmental Groups in Politics. By Philip Lowe and Jane Goyder.

George Allen & Unwin: 1983.

Pp.208. Hbk £15, \$30; pbk £6.95, \$13.95.

OVER two and a half million people belong to environmental groups in Britain; a membership greater than that of any political party or trade union. If you take account of the fact that many people belong to more than one group, you still come up with the astonishing figure that about one in ten of the population is sufficiently concerned about the environment to have joined some association for protecting it.

Nearly fifty per cent of Britain's countryside is covered by some sort of landscape protection policy. The National Trust is the largest private landowner in the country (it owns a quarter of the Lake District and three per cent of the county of Surrey). Green belts, to constrain the indiscriminate spread of bricks and mortar and concrete, occupy eleven per cent of the land area of England and Wales.

These are statements taken from the sober and workmanlike study by Philip Lowe and Jane Goyder. How are they linked? Have the environmental groups brought about a change in social values and government policy? Or are they a "consumer response" to the "marketing" of the environment — clean air and water, national parks, protected areas of natural beauty, habitats preserved for migrating birds, respect for endangered species — manipulated by the media and by politicians? There is no simple answer to these questions.

A good deal has been written about the external relations of environmental groups (for instance, about the part played by the Friends of the Earth in the Windscale inquiry); but very little attention has been given to the internal relations, about how the groups are managed and financed, who decides policy and what precisely is signified by this massive public support. To fill this gap is the purpose of this book, and it does so admirably.

The authors' technique was to circulate a questionnaire to over 80 environmental groups in Britain. It is a technique which natural scientists are apt to regard with suspicion, but Lowe and Goyder dispel such doubts by the scholarly equipment they bring to the work and the judicious

way they have interpreted the responses. They are cautious about drawing conclusions and they offer the reader plenty of material from which to make up his own mind. After a systematic discussion of the organization of environmental groups in national and local politics, they present five case-studies of almost bizarre variety: The Henley Society, the Friends of the Earth, the National

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Dressed to protest — members of Friends of the Earth deliver a letter to 10 Downing Street, urging the Government to ban disposable containers.

Trust, the Royal Society for Nature Conservation, and the European Environmental Bureau.

Who are these two and a half million paid-up members of groups as diverse as the Soils Association, Transport 2000 and the Royal Society for the Protection of Birds? Predominantly (as one is tired of hearing) middle class, but (as the authors say) middle class membership may be characteristic of voluntary organizations of all kinds. Predominantly they are to be found in the affluent and less industrial south east of England. Why do they join? Mainly to register their support — though some groups (e.g. the National Trust) offer perks in the way of free entry and useful booklets — but, with obvious exceptions like the Friends of the Earth, not to get actively involved in the activities of the group. This is consistent with the answer you get when you ask the leaders of the

groups what *they* get out of their membership; it is mainly a source of income, and it is typical of many of the more successful groups that the annual subscription is just about all the leaders want from their members. They run their groups — as some of them admit — as "open oligarchies". Undemocratic as this sounds (and indeed *is*), it makes sense in some groups: it would not be practicable to ask the million members of the National Trust to participate in decisions about the acquisition of estates.

But there are serious drawbacks to passive membership. One is that

the mass following of environmental groups remains a largely untapped political resource ... Though substantially larger than the consumer movement and the women's movement, the environmental movement has won fewer institutional reforms ... its political impact seems likely to be eclipsed by the smaller anti-nuclear movement.

Another drawback is that some admirable groups have lost any openness they ever had, and have become closed oligarchies, run by devoted but inevitably ageing leaders. The Royal Society for the Protection of Birds had the same chairman from 1895 to 1942 and the secretary of the Council for the Preservation of Rural England served for 39 years. The danger of oligarchies, however active they may be, is that government officials and ministers know that they do not really represent the convictions of their membership, only the convictions of their council of management.

And this matters, for the purpose of all environmental groups is to influence officials and ministers. Two main strategies are open to them, and it is the discussion of these strategies that constitutes the most interesting feature of the book. One strategy is to work quietly with no publicity: lunch at the club with the minister or the permanent secretary or a member of parliament, followed by a cautious (oh! so cautious) disclosure of what the government "has in mind" to the secretary of the environmental group, followed by "reasonable and responsible" suggestions from the council of the group. The group achieves consultative status with government officials; it is invited to be represented at discussions before a bill or regulation is drafted; it becomes (to adopt the cynic's verdict) incorporated into the establishment, and thereby rendered innocuous. It may even get financial subsidies for some of its activities.

The alternative strategy is to reach officials and ministers through the public by alerting the media to cases of vandalism on the part of officialdom or neglect or threats to amenity. Sympathetic members

of parliament can be stimulated to ask questions in the House; the appropriate civil servants have to put in hours of work to brief the minister so that he can make a suitably anodyne reply. It is a strategy that organizes campaigns and relies on open confrontation rather than off-the-record meetings with authorities. Its objective is not the cosy consensus but the aroused conscience. The cynic's verdict on this is that what you win after hostility is not as secure as what you win after persuasion.

Both strategies have a part to play in the protection of Britain's environment. If the National Trust were to become militant and abrasive it would be a nonsense, for it has unique privileges (under the Acquisition of Land Act 1946 it can resist the compulsory purchase of any of its estates by a public authority). If the Friends of the Earth were to rely solely on deputations to Whitehall (though they do send deputations and very well informed ones), they would lose their charismatic appeal to some of the "young, well educated, middle-class discontents" (as the book describes them) on whom the organization still relies for most of its support.

Both strategies have proved successful. Low-profile groups are used by members of parliament and even by civil servants to provide expert advice and to act as surrogates for public opinion. High-profile groups, if they have a good case, can make life so uncomfortable for officials that in sheer exasperation some action is taken. Whether the successes have been in the public interest is another question, and one with an ambiguous answer. Elitist amenity groups have been successful in diverting proposed motorways, waste-disposal plants and gypsy campsites from their own neighbourhoods. Because their membership is middle class, it may be that their success is at the expense of the less-privileged members of the community. Urban motorways "tend to go through so called 'soft areas' — areas where opposition and the costs of compensation are least". It is interesting, therefore, to find how, until recently at any rate, environmental issues have not become party-political issues. More spent on protecting the environment means, of course, less spent on some other desirable object, and political parties differ about priorities in spending; but the authors make the significant point that environmental groups work for long-term objectives, must play along with the party in power, and would be unwise to identify themselves with any one party.

One of the case-studies, on the European Environmental Bureau, raises the important question of the international function of the groups discussed in the book. As a first hesitant step towards a United States of Europe, the EEC has an environmental policy (not provided for in the Treaty of Rome) and issues directives intended to "harmonize" environmental policy among the member states. There is a

need, therefore, for an environmental lobby to pace the corridors of the Community's civil service (the Commission) in Brussels. Hence the existence of the European Environmental Bureau, which was conceived after the Stockholm Conference by representatives of the British Conservation Society, the Sierra Club of the USA and the International Institute for Environment and Development. The Bureau acts as a spokesman for dozens of environmental groups in its member countries (who subscribe, not very generously, to its activities). It has already had a beneficial influence on EEC policy, and on the policy in some member states.

This book by Philip Lowe and Jane Goyder is so useful that it would be un-

gracious to wish it had covered even more ground. But how interesting it would be to compare the strategies of environmental groups in the USA and Japan with those of groups in Britain. Robert Boardman (in *International Organisation and the Conservation of Nature*, published by Macmillan in 1981) has covered some of the ground. But a critical discussion of the contrast between the techniques of (say) the Sierra Club and the Council for the Preservation of Rural England, written with the care the authors have put into this book, would be very welcome. □

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The roots of science policy

Harvey Brooks

A Patron for Pure Science: The National Science Foundation's Formative Years, 1945–57.

By J. Merton England.

National Science Foundation,

Washington DC: 1983. Pp.443. \$5.

OFFICIAL histories are often suspect, and run the risk of being uncritical and self-serving in relation to the sponsor. Dr England's book, based on exhaustive research into the National Science Foundation's records and other government documents, is a surprisingly candid, though generally sympathetic, account of the NSF's early years. It takes us from the publication of *Science: The Endless Frontier* in 1945, through the vicissitudes of the legislation in Congress and the final passage of the Act in 1950, the early struggles of the Foundation to formulate its programme and find a place in the sun, ending in 1958 just after Sputnik.

Dr England has wisely chosen to write a thematic history rather than a simple chronological one. He takes a number of issues such as the project grant mechanism, the emergence of big science, the Foundation's evaluative and policy role, the social sciences, and traces the evolution of the Foundation's thinking and policy on each of them. Although the account is analytical and interpretive, it is extremely neutral in tone and it is thus hard to discern the author's own views on any of the policy debates which he describes so meticulously. With a few exceptions there is little praise or blame for any of the major actors; he simply sets forth their views, almost always referenced to specific quotations from the record. There are few villains or heroes, and one does not obtain much sense of the personalities involved. One notable exception to this generalization is

the author's sketch of Alan Waterman, the NSF's first Director who probably did more than any single individual to set the basic patterns and policies of the Foundation and determine its place within the pluralistic organization of American government institutions. Waterman appears as something of a hero, though Dr England does not gloss over his faults. But most of the other *dramatis personae* — household names of American science policy — come through as rather shadowy figures, spokesmen for particular viewpoints or institutional interests, rather than as distinct personalities.

With regard to Waterman there emerges a personality with a strong blend of moderation, even gentleness, and stubbornness — a man who in fact almost always got his way in the end, despite formidable opposition. This characteristic comes through most clearly in the account of the struggle of the Bureau of the Budget to push the Foundation into a more central role in coordinating and evaluating the science-support activities of other federal agencies, particularly the Department of Defense, the National Institutes of Health and the Atomic Energy Commission. With the strong support of the National Science Board, Waterman insisted that the Foundation would not and could not evaluate the work of other agencies, either in basic research or more broadly in applied research and development. This refusal to seize the policy role indicated for the Foundation in the original legislation was not mere bureaucratic timidity but reflected a deeply held view of the nature of science and the scientific community. Waterman believed that "planning" in basic science had to start at the grass roots and could not be masterminded by a scientific bureaucracy. He believed that NSF decisions should reflect the emergence of autonomous consensus within the scientific community itself, and that the most important role of NSF was to catalyse and facilitate the process of consensus formation within the "republic of science".

Closely related to the debate over NSF's coordinating and evaluative role was the question of whether the Foundation should be a "gap filler", whether it should strategically identify and stimulate lagging fields under-supported by mission-orientated agencies, or whether it should become the principal or even the sole patron of basic science throughout the entire federal government. Waterman's position was again one of moderation. He agreed that NSF had a responsibility to ensure the "balanced" development of science as a whole, but refused to confine NSF purely to gap-filling, feeling that it should have a presence in all principal areas of the natural sciences. At the same time, he was diffident about taking over programmes in basic research from other agencies as he was being urged to do by the Bureau of the Budget. This intermediate position between gap-filler and principal patron of basic science is one that has persisted to this day.

Many of the issues and debates described by Dr England persist to this day. They are the perennials of science policy — geographical distribution and institutional development versus the support of excellence; federal support of the basic social sciences; the project grant system with peer review versus other modes of support; the determination of priorities; the proper balance between big science and little science; and payment of institutional costs such as faculty salaries and overhead costs from research grants. Other issues which loom large in this history have mercifully disappeared — loyalty oaths or loyalty investigations for fellowship recipients or grant recipients; the desirability of fostering international scientific communication; the role of NSF in defence-orientated research; presidential appointments of the Director; and patent policy.

For those of us who lived through the history of the NSF and the numerous science policy debates of the past 30 years, this book is a sentimental journey. It is a remarkable story, particularly so in the general context of the American political system. The book will remain an indispensable source book for future historians and analysts of American science policy. One can also not fail to be impressed that so many contemporary debates are not new, but have a venerable history.

Readers will look forward with great eagerness to the planned future volumes in this series, which will carry the story up to date. Yet I believe this volume will remain the most interesting simply because it contains the roots of the later events and developments, which were mostly elaborations and refinements on trends that were already apparent in the Foundation's early history. □

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All in theory — the analysis of music

Christopher Longuet-Higgins

A Generative Theory of Tonal Music.

By Fred Lerdahl and Ray Jackendoff.
MIT Press: 1983. Pp.368. \$35, £31.50.

FOR SOME time it has been rumoured in music-theoretical circles that a definitive theory of music had at last been formulated by the Chomskyan linguist Ray Jackendoff and the composer Fred Lerdahl. Their book would redefine the field of music theory, and make all previous work seem as antediluvian as pre-Chomskyan linguistics after the publication of *Syntactic Structures*. Now, at long last, *A Generative Theory of Tonal Music* emerges from the MIT Press, adorned with wide margins and expensively engraved musical illustrations from the works of the very best classical composers. The authors, so the flyleaf announces, "collaborate here on a seminal theory synthesizing the outlook and methodology of contemporary linguistics with the insights of recent music theory".

It would be impossible to gainsay the worth of such a project, but like so many books on musical theory this one woefully disappoints the expectant reader. It adopts the methodology of contemporary linguistics only in so far as the earliest and latest chapters (due largely, one suspects, to Jackendoff) belabour the reader with principles of theory construction and claims to generality which are quite unjustified by the detailed contents of the central chapters. "Recent music theory" apparently comprises a series of "preference rules" of almost unbelievable vagueness for reducing a piece of homophonic music — or rather, its printed score — to a single tonic chord, in the manner of the early twentieth-century theorist Heinrich Schenker.

The only sense in which the theory might be described as "generative" is that it permits the analyst to erect above the notes of a musical score a flimsy tree of dependency relations, but the authors appear not to notice the connection between their ideas and those of dependency grammar. They are uncomfortably aware of the formal imprecision of their preference rules, but they elevate into a positive virtue the evident impossibility of incorporating them into effective procedures for the structural description of "musical surfaces" — a term which they use freely but without formal definition (it does not even appear in the index). As for the word "tonal" in the title, the book is utterly barren of any new ideas on tonality; as the authors say on page 117:

In what follows we can take as given the classical Western tonal pitch system — the major-minor scale system, the traditional classifications of

consonance and dissonance, the triadic harmonic system with its roots and inversions, the circle-of-fifths system, and the principles of good voice-leading. Though all of these principles could and should be formalized, they are largely idiom-specific, and are well understood informally within the traditional disciplines of harmony and counterpoint. Nothing will be lost if we conveniently consider them to be an input to the theory of reductions.

It does not seem to occur to them that an elementary obligation on any theory of tonal music (in the sense in which they use that term) is to examine whether the principles they mention are indeed as well understood as they claim, or whether some of these principles (such as the circle-of-fifths system) actually do violence to the conceptual basis of tonality. If, furthermore, the tonal principles to which the authors appeal in their theory of reductions are indeed so idiom-specific, how can one be sure that the theory has any application outside the Western tonal tradition?

The detailed discussions of "group structure" and "metrical structure", and the related processes of "time-span reduction" and "prolongational reduction" are unsatisfactory in a different way; not only does the latter process look uncomfortably like a remedy for the deficiencies of the former, but it could not even in principle be applied to contrapuntal music such as fugue. Moreover, the relevant parts of the theory do not seem to accommodate in any natural way such important concepts as rhythmic ambiguity or syncopation; for the concept of syncopation, this is the best they can supply in the way of a formal definition (p.77):

In general the phenomenon of syncopation can be formally [*sic*] characterized as a situation [*sic*] in which the global demands of metrical well-formedness conflict with and override local preferences.

Lerdahl and Jackendoff are, it seems, in favour of constructing a formally precise theory of music, in principle but not in practice. In point of fact much of the necessary groundwork has been done by other theorists in recent years, particularly in England; but of this work the authors seem to be quite unaware, although it would enable them to clarify their own thoughts considerably. One cannot help feeling that in view of the very limited range of application of their present ideas it would have been better to make more modest claims for what they have so far achieved.

In spite of all its faults, however, the book will undoubtedly provoke thought, especially among psychologists of music, as to what a theory of tonal music would look like if somebody were actually to produce one. □

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Peeling apart nature

George Sugihara

Hierarchy: Perspectives for Ecological Complexity.

By T.F.H. Allen and Thomas B. Starr.
University of Chicago Press: 1982.
Pp.310. \$27.50, £22

IT IS somehow ironic that while the sciences of the very small (e.g. atomic physics and chemistry) and of the very large (e.g. astrophysics) have achieved such great success, a middle-sized phenomenon such as the ecology of the natural world has been so elusive to scientific scrutiny. From the human vantage point, ecological systems appear unmanageably complex. To what extent could this complexity be a consequence of our anthropocentrically scaled perceptions? Of more practical importance, how should the investigator resolve interdependent ecological systems into manageably small pieces without destroying the complexity of the whole?

As Allen and Starr tell us, the beginnings of an answer to these questions are now coalescing into a set of ideas known collectively as hierarchy theory. The term "theory" is used liberally here, in that there is as yet no systematic statement of rules or principles to be followed. Nonetheless, as energetically reviewed in this book, there is a definite body of problems and insights related to scaling phenomena that form the basis of current thinking on hierarchies.

In its most general sense, a hierarchy is defined as a partial ordering of a set of objects. Herbert Simon, a founding spokesman for the field, uses the example of a nested set of Chinese boxes to illustrate how the relation "contained in" may generate such an ordering. Similarly, in an ethological context the relation "dominates" may generate a hierarchy in dominance relationships. Although a given set of objects may or may not be hierarchical depending on the ordering relation chosen, there is reason to believe that the natural world is organized hierarchically in an ontological sense. This follows from the argument that systems built in such a manner, from stable parts of intermediate complexity, tend to evolve much faster than those constructed at once.

The challenge posed by this viewpoint is to find a natural ordering relation and a means for discerning discrete objects or levels for such systems. Are the categories individuals, populations, communities, ecosystems reasonable object levels? If so, what is the meaningful ordering relation? Or conversely, given a meaningful ordering relation can one discern discrete object levels? Simon suggests that natural frequency might be an important ordering relation. Thus cells operate at a higher frequency than individuals, which in turn cycle faster than populations etc. Allen and

Starr suggest that "constraint" loosely defined might be important, with higher hierarchical levels constraining lower ones.

The philosophical framework for dealing with hierarchies falls roughly under the heading of dialectical materialism. It differs from the single-level view offered by reductionism in that the whole is regarded as a contingent structure which is understood in terms of the simultaneous action of several different levels of organization. To understand the whole, one must understand interactions between the various levels in the structure. This viewpoint was enunciated recently by Stephen Gould (*Science* 216, 380-387; 1982) as a framework for studying evolution. Natural selection, he advocates, should be viewed as operating simultaneously on levels above and below the individual, from gene through clade. It is the interactions between levels that shape the path of evolution. Thus, for example, benefits to the individual gained by specialization may be offset by its cost to the species. Such negative and positive effects between levels may be responsible for stability and change in evolution.

On the more tactical side, hierarchy theory attempts to embrace problems

related to scaling in making observations and measurements. As pointed out by Allen and Starr, while algal ecologists are willing to consider time frames which span multiple generations, for terrestrial ecologists the time frame usually includes only a fraction of the life span of a single tree. Thus our anthropocentric scale as investigators may introduce a kind of observational parallax. Whether we are willing to accept communities as distinct objects or blurred continua may depend on the grain of our observations.

Given the scope and novelty of the subject, Allen and Starr have attempted an ambitious task in drawing together many of the threads of thinking on hierarchies. They present this in a very personal synthetic style with wit and zeal, incorporating jargon and concepts from systems' science, statistics and science fiction. Although the authors overstate their position somewhat, this book should fill an important role if it helps to stimulate a future systematic and rigorous treatment of hierarchies. □

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Feast of proteins

Peter D.G. Dean

Protein Purification: Principles and Practice.

By Robert K. Scopes.
Springer-Verlag: 1983. Pp.282.
DM79, \$31.60.

AN HOUR or two in the company of genetic engineers might lead you to believe that protein purification has no place in bioengineering. By contrast, I have felt for some time that the need to isolate ultra-pure cloned proteins from cell pastes has become an urgent problem and see it as a bottleneck in the manufacture of such proteins.

In *Protein Purification*, Robert Scopes demonstrates his skill as a practitioner of purification techniques *par excellence*. He attacks and analyses his subject (and incidentally provides answers for the bio-engineer at the same time) with clear and considered opinions in a manner which is instructive to the beginner, reassuring to the experienced bench worker and thought-provoking to the supervisor. I found this short book a feast and a genuine pleasure to read. The delightful, chatty style reminds one nostalgically of yesterday's undergraduate tutorials.

The growing range of protein purification methods is presented with a refreshing mixture of interesting theory (a rare quality) and a wealth of practical data and practical tips. Thus the section on affinity elution begins with four pages of

introductory theory, continues with a page of practical steps, five pages of more theory and ends with a page of applications. This section is immediately followed by 20 pages on affinity adsorption of which a third is devoted to dye ligand chromatography.

The organization of the chapters takes the reader from "the enzyme purification laboratory" through "making an extract". The latter chapters make up an introductory section which is followed by a series of chapters on separation techniques, precipitation, adsorption and separation in solution. Perhaps one of the most useful set of chapters is the final four which include accounts of maintenance of enzyme activity, optimization procedures, measurement of enzyme activity and analysis of purity.

Errors are remarkably few and the layout is attractive; the author is to be commended particularly for his clear diagrams. The few trivial omissions that I noticed were recent developments in solid-phase ampholytes, high-performance liquid chromatography, the use of detergents in dye ligand chromatography, affinity gel filtration and the more recent work from Wilchek's laboratory on the mechanism of the cyanogen bromide activation reaction.

This excellent text is a must for all undergraduates wishing to learn about protein purification. It should also be on every laboratory shelf where proteins are being handled or purified. □

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Model solutions to energy forecasting

M.R. Alexander

Energy Strategies for the UK.

By S.C. Littlechild and K.G. Vaidya.
George Allen & Unwin: 1982. Pp.232.
£15, \$29.95.

"THOSE who foretell the future lie even when they tell the truth" — in the light of this truism from the Koran, what credibility can forecasters hope to achieve? *Energy Strategies for the UK* describes the mechanism formulated by its authors to look into the future — the Birmingham Energy Model (BEM), a linear-programme-based optimization model — using "current" estimates of future costs, details of environmental constraints in the United Kingdom and views on fuel prices.

The BEM constitutes a laudable attempt on the problem of energy forecasting, but perhaps so difficult a task is best not dealt with by so rigorous an approach. Optimization must be directionally correct but we know so little of the future that any optimum is likely to be highly erroneous and debatable. In addition, the 1982 world energy scene looks so different from its 1977 counter-part which provides the basis of the book.

Besides the numerous uncertainties which the authors analyse in Chapters 6 and 7 (including levels of oil and gas reserves, different oil and gas price profiles, variations in the terminal value of fuel reserves, discount rates, security of the UK's oil supply, and fluctuations in the UK's balance of payments and level of coal imports) the model has to assume a detailed knowledge of other aspects of the future. One further area of uncertainty must be technology; another the interrelationship, on an international scale, between fuel discovery rates, relative prices and inter-fuel substitution; and a third political direction.

As to technology, a primary consideration is the potential of synthetic oil and gas. That potential depends upon production costs. In 1982 the IEA stated in their paper *Coal Liquefaction — A Technology Review* that

the product cost from a full scale coal liquefaction plant is likely to be between US \$60 and US \$100 per barrel in 1982 prices. Since no full-scale direct coal liquefaction plant (the major single cost component) has been built, even the most careful estimates have some uncertainty. A full detailed engineering, design and costing study for a specific coal, process and site would cost of the order of US \$100–200 million.

If this was the state of affairs last year, what faith can we place in results generated from 1977 estimates; or in 1990 on the 1982 forecasts? And can we believe the authors' assertion (p.134) that an optimal solution

involves using coal to make synthetic oil whilst simultaneously oil is used in the production of peak-saving electricity?

Fuel prices, the basic model determinant, hide enormous utilization differences. Real energy price comparisons are difficult to make, as they include both the energy and the "quality" components (e.g. calorific content and the storage, handling and exhaust gas scrubbing costs of coal). Many American companies (General Motors, for instance) have found the technical advances in the use of electricity overcome its apparent regional (3:1) price disadvantage to natural gas. Typical preferred electricity uses now include paint and ink applications, electric induction furnaces to melt metals, microwave dryers and lasers for metal treatment.

Politics complicate matters even further; some current imponderables include the

effects of EEC acid rain regulations, US pressure to reduce Russian gas exports and, more parochially, the retention of uneconomic coal mines and industrial restructuring.

In *Energy Strategies for the UK* the authors illustrate through their model a range of energy options, and the book will provide a useful basis for student discussion. However, although the authors rightly provide a variety of solutions, they give little guidance towards identifying the "most desirable". With oil production results gyrating up to 100%, as seen in Chapter 6, readers would have profited from some probability analysis of the input parameters and the identification of the median or recommended result. □

M.R. Alexander is a member of the British Petroleum Company's Policy Review Unit, London.

Stress on crystals

H.G. Drickamer

Comparative Crystal Chemistry:

Temperature, Pressure, Composition
and the Variation of Crystal Structure.

By Robert M. Hazen and Larry W. Finger.

Wiley: 1983. Pp.231. \$43.95, £19.50.

THIS is a gem of a book! It is divided into two parts of approximately equal length, the first of which covers the technical problems of studying crystal structure at high pressure.

The discussions here are complete, thorough and clear, those on the various types of diamond anvil cells currently in use being especially well done. One can get a real feel for the techniques, methods of construction and problems in using the cells, as well as for the applications of various designs. Every aspect of the topic is included except a set of dimensioned engineering drawings, and this lack is partially compensated for by a list of vendors. The account of how to add the variable of high temperature is equally complete. In each case the authors clearly outline the advantages, limitations and possibilities.

In the final chapter of this section one is given a reasonably detailed treatment of how to extract information from the X-ray measurements, together with a program for calculation of strain tensor from unit cell measurements. It is not intended that one could learn X-ray crystallography from scratch from the book but the discussion is most helpful.

The second half of the book deals with structures and lattice parameters as affected by temperature, pressure and composition. The emphasis is overwhelmingly on ionic model compounds, especially oxides, and on the more complex silicates found in the Earth. Within this

rather restrictive limitation, however, the results presented are remarkably complete. A wide variety of largely empirical relationships involving lattice parameters and elastic or thermodynamic functions are presented, and there would appear to be here a rich source of information for the theorist.

Comparative Crystal Chemistry will be a "must" for both geochemists and geophysicists. It is written so that either an experienced practitioner or a student can use it. In addition, much of the material should be helpful to solid state chemists as well as some solid state physicists. □

H.G. Drickamer is Professor of Chemical Engineering and Physical Chemistry at the University of Illinois, Urbana.

Useful neurohormones

Larry L. Keeley

Insect Neurohormones.

By Marie Raabe.

Plenum: 1982. Pp.352. \$42.50, £29.75.

MARIE Raabe has undertaken the heroic task of trying single-handedly to bring order to a large body of information that has not previously been comprehensively reviewed. During the past 20 years, most research reports, books, review articles and symposium proceedings dealing with insect endocrinology have focused on the ecdysones and juvenile hormones, with only cursory attention being given to the neurohormones and their regulatory effects. As the author makes clear, however, neurohormones exert profound effects on a wide variety of physiological processes in insects. The recent upsurge of work on vertebrate neuroendocrinology and neurobiology has led in addition to a growing interest in insect neuroendocrin-

ology. Therefore, a book on this topic is relevant at present to consolidate the existing information.

It is no fault of Raabe's that her monograph clearly illustrates the lack of definition inherent in insect neuroendocrine research. Except for proctolin and adipokinetic hormone, the insect neurohormones have generally not been purified and their chemical structures remain unknown. Hence, unlike authors of texts on vertebrate endocrinology, who are able to discuss defined hormones with specific actions, Dr Raabe has had to consider insect neurohormones largely from the standpoint of physiological responses to putative factors present in crude extracts of neurohaemal tissues.

In these circumstances the book's principal merit is in providing a summary of the large quantity of research literature available on the topic. Unfortunately, the information is presented largely in the form of a précis of research results which often leaves the reader bewildered by the array of facts. Topics reviewed range from neurosecretory cells and neurohaemal structures

to evidence for the existence of specific neurohormones and their physiological effects. An addendum brings the reader abreast of significant findings that appeared during the preparation time needed for publication.

There are many useful diagrams, figures and tables that help to clarify the textual material by illustration or that summarize the diverse endocrine situations found among insect species. Some figures are overly complex with inadequate captions, however, and I found the method of reference citation, by listing authors and dates throughout the text, rather distracting.

Research libraries and researchers specializing in comparative endocrinology or insect physiology will undoubtedly find the book useful. As a first effort it will probably not be the definitive account of the subject, but it will prove valuable for bringing the serious student of insect neuroendocrinology abreast of events in this rapidly-developing field. □

Larry L. Keeley is Professor of Entomology at Texas A & M University College Station, Texas.

Meeting of parasites

R.J.M. Wilson

Malaria and the Red Cell: Ciba Foundation Symposium 94.
Pitman/Ciba Pharmaceutical: 1983.
Pp.257. £25, \$35

IN APRIL of last year, some 30 parasitologists and biochemists came together in London to exchange ideas on "malaria and the red cell". The theme of the symposium was how the malaria parasite recognizes, invades, catabolizes, alters and finally destroys the red cell. The discussions are recorded in this commendably readable volume, which is both wide-ranging in content and stimulating in that it raises as many questions as it answers.

Invasion of the red cell commences when the merozoite surface coat interacts with red cell membrane sialoglycoproteins. The glyophorins on human red cells are firmly established as specific recognition sites for *Plasmodium falciparum*, but different structures are required for other malaria parasites. Rare types of red cells, deficient in or with modified glyophorins, have been used to assess the effects on invasion of surface charge, oligosaccharide configurations and polypeptide structures proximal to the bilayer.

Apposition of the apical region of the merozoite to the red cell membrane triggers an active invasion process: a localized junction zone is formed within which fine fibrils span a 10 nm gap between parasite and red cell, an electron opaque material is extruded from the parasite and the inner leaflet of the red cell bilayer assumes an increased electron density. As recorded in the book,

chief among various speculations is that a strongly cationic parasite protein is inserted into the inner leaflet of the bilayer destabilizing it and dissociating the cytoskeleton so that endocytosis of membrane ensues. These events culminate in the encapsulation of the parasite within the red cell.

The resistance of some hereditary variants of red cells to invasion, as well as changes in susceptibility associated with red cell ageing, can be explained plausibly by this new information. However, it is curious that in the three most prevalent genetically determined pathological conditions of human beings, which have all been related to malaria — sickle cell anaemia, the thalassaemia syndrome and glucose-6-phosphate dehydrogenase deficiency (G-6PD(-)) — selection is probably not exerted primarily at the level of red cell invasion. Rather, invasion is rendered abortive by failure of intracellular parasites to mature.

In parasitized sickle cells, the erythrocyte membrane sustains sickling damage under low oxygen tensions and decreased intracellular pH. The protective mechanism in G-6PD(-) is more paradoxical since the Gd^A gene seems to protect heterozygous females (cellular mosaics), but not male hemizygotes (whose cells are uniformly deficient). The sensitivity of thalassaemic and G-6PD(-) cells to oxidative stress has been seized upon as a major mechanism of resistance, since hydrogen peroxide or reactive intermediates of oxygen can decrease parasite multiplication *in vitro*. This hypothesis remains controversial but had several supporters at the symposium and will doubtless be pursued.

Elegant electron micrographs illustrated

in the book show how maturing intraerythrocytic parasites modify the red cell membrane in many other ways. "Knobs", clefts and caveolae play a significant role in sequestering infected cells on the venous endothelium and may alter the transport of ions and metabolites into parasitized cells: the intraerythrocytic Ca²⁺ content, for example, increases 10–20-fold (> 90% within the parasite). The induction of new permeability pathways for amino acids and other substrates required by the parasite have an additional significance — the potential for chemotherapeutic effects at the level of the red cell membrane; anti-malarials such as chloroquine are believed to form toxic complexes within the food vacuoles of the parasite itself where haemoglobin is digested.

Antigenic changes which arise from the insertion of parasite components into the red cell membrane, as well as by the exposure of previously cryptic isoantigens, are described in several sections of the book. The relevance of autoimmune recognition of red cells in immunopathology and in the induction of immunity to malaria is at present unclear. In patients with anaemia following infection with *P. falciparum*, the role of immune haemolysis seems to be small, but a gross diserythropoiesis was reported at the meeting without identification of the mechanism. At present there is no logical approach to the management of such patients.

Substantial changes also occur in the microcirculation of the spleen during malaria infection. The altered rheological properties of infected red cells contribute to splenic trapping, and cordal macrophages may kill parasites by direct contact or by soluble mediators. Exacerbation of malaria in first pregnancies might be due to parasite sequestration in a new blood reservoir, the placenta, thereby obviating protective mechanisms in the spleen. Another fascinating interaction discussed at the symposium was the modulation of the expression of parasite surface antigens by the spleen. When introduced into a splenectomized host, parasites of an antigen-positive phenotype switch to a negative phenotype due to altered expression by the entire parasite population. The parasite's ability to express alternative molecules on the surface of the red cell may be of adaptive value in the face of spleen-mediated host immunity.

Although vaccines were not widely discussed at the symposium, it is clear that efforts to elucidate the molecular basis for events which either sustain or curtail parasitization of red cells by malaria parasites have gathered considerable momentum. The growing input from the new technologies gives grounds for further optimism. □

R.J.M. Wilson is in the Division of Parasitology at the National Institute for Medical Research, Mill Hill, London.

Who needs monoclonals?

Karol Sikora looks at monoclonal antibodies, the rising stars of the biotechnology era.

SINCE their discovery in 1975, monoclonal antibodies (MCAs) have become the key research tool of the biotechnology era, opening up many new fields of biological and clinical research. They exploit the remarkable specificity of the immune system — nothing new in itself since immunological techniques have already harnessed it for many years. What MCAs do bring to research, however, is a degree of specificity which has previously been impossible. Polyclonal antisera, produced by animals, have been used for many years to identify, purify and assay a wide range of molecules including enzymes, drugs and hormones, as well as to provide markers for several different cell types. However, they are subject to batch-to-batch variation between sera taken from different animals at different times. Many investigators know that sinking feeling that occurs when an animal, whose serum forms the corner stone of an assay, dies.

In contrast, MCAs, which are produced by single clones of antibody-producing cells, can be extracted with great purity and in unlimited quantities. If necessary, they can be prepared to complete purity by growing hybridoma cells in serum-free media and then purifying the immunoglobulin that they produce from large quantities of supernatant. Many such antibodies are now becoming routinely used in industrial purification systems as well as in clinical assay laboratories.

Identifying cells

One area in which MCAs have made an essential contribution is in reaching our present understanding of lymphocyte differentiation. Many MCAs are available to precisely identify those lymphocytes which are involved in health and disease. Now both T and B cells can be simply and accurately quantified; subtypes of T cells, such as helper and suppressor populations, identified; and the immunoglobulin chains being secreted by B lymphocytes, and those expressed on their surface, detected. Such assays may prove useful in identifying a variety of immunological diseases as well as in the monitoring of patients undergoing immunosuppression for organ transplantation.

Currently, the ability of MCAs to identify particular cells is finding a variety of clinical and laboratory uses. Anti-T lymphocyte antibodies are now used to destroy T cells in donor bone marrow prior

to transplantation, which in turn can prevent the development of graft versus host reaction in the transplant recipient. This occurs when the viable T cells in the donor marrow recognise the "foreign" histocompatibility antigens on the cells of one host and start to destroy them. Another application arises from the coupling of the rapidly-advancing technology of flow cytometry with a growing battery of MCAs which is now making it possible to analyse and sort mixed populations of cells. Such analyses produce tremendous amounts of information and yield further insights into the complexity of the immune system. Polyclonal antisera, with all their inherent problems, are still being used for determining blood groups and for tissue typing, that is to identify the molecular polymorphisms that occur not between cells but between individuals. However, panels of MCAs are becoming available that will do this more rapidly and precisely in routine laboratories.

MCAs and cancer

Many investigators have searched for MCAs that specifically recognise tumour antigens and thus can identify cancer cells, but their success has so far been limited. It may well be that the human cancer cell surface differs from its normal counterpart only in the quantity of the individual components of its molecular display. If this is so, then the search for a specific tumour antigen will prove elusive.

The most immediate use of MCAs for diagnosis is in detecting circulating markers in the peripheral blood, such as α -fetoprotein and human chorionic gonadotropin in testicular cancer and carcino-embryonic antigen in colorectal cancer, in the peripheral blood. Such products are shed by tumour cells, and by assaying them accurately it is possible to assess the tumour load of an individual patient.

MCAs are also set to bring a mini-revolution to the histology laboratories. Using immunohistology, pathologists will at last be able to identify precisely the tissue of origin of a tumour or other disease process, something which is difficult to achieve with present techniques. The easy detection of cancer cells in smears prepared from body secretions such as sputum or vaginal mucus could rapidly lead to automated cytological diagnosis, thus reducing its cost and making it available to a wider range of

patients. The use of MCAs tagged with radioactive isotopes and administered to patients has already been shown to localize tumours. Further antibodies will be developed that allow the precise localization, and perhaps destruction, of tumours in patients.

Diagnosis and purification

In infectious disease it is becoming increasingly important to make a rapid diagnosis without the delay involved in waiting for a culture of microorganisms or viruses to develop. The supreme specificity of MCAs permits a wide variety of bacterial and viral organisms to be screened directly by examining pathological tissues. Similarly a variety of indicators of disease can be easily identified in patients serum using MCAs. These indicators include tissue-specific enzymes released by damaged heart muscle and liver which can be measured by radioimmunoassay. To ease the difficulties of introducing rapidly advancing technology into routine laboratories, several manufacturers have now produced kits of reagents based on MCAs. Such kits provide all the reagents required for specific assays, often with some form of quality control.

As well as providing for diagnosis, MCAs aid in the purification of a wide variety of antibodies and drugs. Interferons, for example, are purified using affinity columns to which anti-interferon antibodies have been bound. It is only in this way that sufficient quantities of interferon have become available for widespread clinical trials in infectious diseases and cancer.

It is now quite likely that MCAs against every possible antigenic determinant have already been made and lie dormant in laboratory freezers around the world. The searching and screening involved in matching an antibody to its antigen is an exhausting but rewarding process. Antibodies that have proven of use in the detection of blood groups have come by trying to make antibodies to cancer cells. Gene transfer techniques will soon release a second generation of antigen-targeted MCAs on an eager research community. Their impact in clinical and industrial laboratories will be considerable. □

Dr Karol Sikora is the director of the Ludwig Institute for Cancer Research, Cambridge, UK.

Monoclonal reagent-kits and tests

● Lympho/Scan anti lambda is a new immunoperoxidase staining kit that has been introduced by Biomedica for the detection of human lambda light chain in histological sections of lymphatic and other tissues. The identification of the distribution of cytoplasmic lambda light chain in lymphoid tissues is useful in the differentiation of monoclonal from polyclonal B cell proliferations. It also reduces the number of incubations and reagent preparations required, and its accelerated procedure reduces the reagent incubations to 5–10 minutes for the lambda specific antibody followed by two 5 minute incubations for the peroxidase reagent and the chromogen developer.

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● A new RubEnz M enzyme-immunoassay kit for IgM-class antibodies against rubella is available from Northumbria Biologicals. The assay uses an IgM-class antibody capture technique. It has been estimated that the test will detect IgM antibodies from approximately 1 to 4 weeks after the onset of illness. In addition to the RubEnz M kits, Northumbria Biologicals also supply the monoclonal anti-rubella antibody, both unconjugated and as a peroxidase conjugate.

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● A test which identifies chlamydiae using monoclonal antibodies is now available from Biosoft, either for immunoperoxidase testing or for indirect immunofluorescence. The test will identify chlamydiae either in cell culture or from a swab sample. The indirect immunofluorescence kit includes 5 ml of anti-chlamydia monoclonal antibody, and, for the peroxidase-anti-peroxidase identification test, 5 ml of mouse anti-IgG (H + L) antibody, 5 ml of mouse peroxidase and also anti-peroxidase.

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● Promega Biotec is now manufacturing a single-tube, gamma-³²P-ATP synthesis system. Called the GammaPrep-A reaction system, the product simplifies the process of making labelled ATP. Promega Biotec is also offering a GammaPrep-G system for the synthesis of gamma-³²P-GTP, a GammaPrep-U system for gamma-³²P-UTP, and a GammaPrep-C system for gamma-³²P-CTP. Promega Biotec, also produces RNasin ribonuclease inhibitor, Packagene *in vitro* lambda DNA packaging systems, rabbit reticulocyte lysate, Topo I wheat germ topoisomerase I, and restriction endonucleases, including Sca I and Sin I.

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● Coulter has recently introduced a new T and B kit which is suitable for use with flow cytometers or for microscopy. Also available from Coulter are specific B cell markers, B1 and B2; a specific monocyte/macrophage marker, MO2; a specific J5 (CALLA) marker; activation markers such as I2 and ILR2; and immunoglobulins including IgG, IgD and IgM. The Coulter Clone range at present includes the following monoclonal antibodies: T11A, T8A, T6A, T4A, T1B; B1, B2; I2, ILR2; MO1, MO2; J5; IgD, IgG, IgM, kappa and lambda.

Circle No. 104 on Reader Service Card

● Celltech Diagnostics has announced the release of its first immunoassay product. This is an immunoradiometric assay kit for the measurement of serum interferon- α levels. The Sucrosep interferon assay is based on a monoclonal antibody to interferon- α and incorporates a non-centrifuged separation system which increases the sensitivity of the test. Replacement of the currently-used bioassays with the Sucrosep interferon assay reduces assay time from several days to 4 hours and gives a sensitivity of 1 unit per ml. Each assay kit contains the reagents and plasticware to carry out 120 determinations.

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● The new IgE FAST (Fluor Allergo Sorbant) test from Dynatech Laboratories provides a rapid test for hay fever and common allergies, giving results in 4½ hours. The speed is obtained by using a fluorescent microplate reader which can read 96 samples in 30 seconds, and with a different allergen per well, this can test up to 96 allergens. The tests provide qualitative as well as quantitative measurement of specific IgE in the blood. Around 100 standard IgE FAST test kits are available from Dynatech, including common grasses, animal allergens and mites.

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Monoclonals for medical research

● A new method of detecting and classifying tumours has been developed by Labsystems of Finland. The new method uses monoclonal antibodies to intracellular structural proteins. These structural proteins, or cytoskeletal filaments, are present in all nucleated cells and are specific to a particular cell type. This means that, using the monoclonal antibodies, the tumour type can be reliably determined from a metastasis. Labsystems have developed monoclonal antibodies to the cytoskeletal filaments of all cell types. These monoclonal antibodies attach to the filaments and can then be identified using, for example, fluorescent conjugate markers.

Circle No. 107 on Reader Service Card

● A new series of reagents for ABO blood grouping has been launched in the UK by Celltech. The reagents are monoclonal antibodies, an anti-A and an anti-B, which distinguish the major blood groups, O, A, B and AB. Celltech plans to introduce monoclonal antibodies for other blood grouping applications within the next two years.

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● The anti-transferrin receptor antibody is a new research monoclonal antibody (clone L01) from Becton Dickinson which detects the human transferrin receptor (M_r 180,000). The antigen is found on all mitogen and allo-antigen activated lymphoblasts, most human tumour cell lines, and normal peripheral blood monocytes. The anti-transferrin receptor antibody is valuable for the detection of activated lymphocytes and for the analysis of cellular proliferation. The product is available as either purified antibody or fluorescein conjugate.

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Becton Dickinson's anti-transferrin receptor.



Monoclonal antibodies from Labsystems.

● Two new monoclonal blood grouping reagents are being introduced by Lorne Laboratories — monoclonal anti-A and anti-B. Derived from murine hybridoma cells, the antibodies are IgM complete. Mouse monoclonal antibodies do not give false positive results and therefore it is not critical to read them within 2 minutes. However peripheral drying should not be mistaken for agglutination after this time. The basic material will be produced by Celltech and this will be reprocessed by Lorne. The antibody has a shelf-life of one year, dependent on storage at 4°C.

Circle No. 110 on Reader Service Card

● Biosoft have released a new mouse monoclonal antibody specific for type I herpes simplex virus. It is of the IgG1 subclass and the specificity, determined by enzyme immunoassay, showed that there was no cross reaction with the type II herpes simplex virus. The antibody reacts with a viral cytoplasmic protein of M_r 80,000 and it does not neutralize even at concentrations ten times greater than that recommended. It is derived from the fusion of Sp20 mouse myeloma cells with BALB/c immunized mouse splenocytes of the VR3 lineage. The antibody is derived from partially purified ascites and should be stored at 4°C or -20°C, avoiding repeated removal from refrigeration. Its applications include the characterization of herpes simplex virus type I by indirect immunofluorescence or enzyme immunoassay.

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● Biosoft have produced a mouse IgG2a (kappa light chain) anti-human IgM Fc receptor monoclonal antibody. The antibody, called TIBI-82, is derived from the fusion of SP₂O mouse myeloma cells with BALB/c mouse splenocytes after immunization with human heavy chain. It is purified by ammonium sulphate precipitation and DEAE-cellulose chromatography. The TIBI-82 antibody can be used to detect rubella, syphilis and hepatitis A and B in standard immunoassays.

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● Now available from Sera-Lab is a monoclonal antibody (MAS 079) which reacts with 20–25% of peripheral blood cells, including B lymphocytes and monocytes, B lymphoblastoid lines, myeloblasts in bone marrow and thymic epithelium. It is cytotoxic to more than 90% of HLA-DR⁺ cells in human peripheral blood and bone marrow in the presence of human and rabbit complement and has been used in fluorescence-activated cell sorting (FACS) and cytotoxicity assays to demonstrate the presence of Ia-like antigens on bone marrow progenitor cells, including pluripotent haemopoietic progenitor cells.

Circle No. 113 on Reader Service Card

● Biogen has announced the shipment of its first commercial product produced through recombinant DNA technology. The product, hepatitis B core antigen, is being sold to the Green Cross Corporation of Osaka, Japan, under a supply agreement. Green Cross will use the antigen in diagnostic kits to test for the presence of hepatitis B in human blood. Biogen purifies the bacterially-produced core particles and sells them as bulk material. The core antigen is then assembled into blood testing kits to test for the presence of antibodies against the core material to determine whether or not the blood donor has been infected with hepatitis B virus and might be a carrier of the disease. Currently, blood used in blood banks is tested for the presence of the surface antigen of the virus or for antibodies directed against the surface of the virus particle to detect a previous infection. As vaccines against hepatitis B, made from the surface antigen isolated from human blood, come into use the anti-surface testing kits will begin to yield false positives. The anti-core kits will not suffer from this defect and also should be more sensitive.

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Monoclonal antibodies for research

● Affinity purified rabbit immunoglobulins to human interferons are now available from Interferon Sciences. Alpha and gamma interferon specificities can both be obtained, each of which contains only detectable levels of IgG, and neither of which shows any reactivity with other human interferons. The immunoglobulins are purified using goat- and anti-rabbit immunoglobulin. They can be used in the detection and assay of human interferons using techniques such as radioimmunoassay, fluorescent immunoassay or ELISA, or in the purification of human interferon.

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● Multi-clone anti-Leu-3a + 3b is a new reagent from Becton Dickinson for localizing human helper T cells in frozen tissue sections. This research reagent combines two independent monoclonal antibodies to the Leu-3 antigen (M_r 55,000). When used in an indirect immunoperoxidase procedure, the multi-clone reagent gives membrane staining of T helper cells, and cytoplasmic staining of tissue macrophages. The antibodies are provided in a 100-test dropper bottle and the tests consist of one drop for each frozen section. The reagent is in a stable solution and may be stored for up to twelve months.

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● Anti-Leu-12, a new monoclonal antibody which detects human B cells in blood and tissue, is now available from Becton Dickinson. This antibody recognises a unique antigen which is found on all B cells, as defined by the presence of kappa or lambda light chains. Anti-Leu-12 has not been found to react with normal T cells, Con A-stimulated T cells, or granulocytes, and has only marginal reactivity with monocytes and tissue macrophages. The reagent is provided in a purified form and also as a fluorescein conjugate for direct immunofluorescence studies. Both reagents are available in solution as 100-test vials.

Circle No. 120 on Reader Service Card

● A mouse monoclonal antibody against herpes simplex virus type I and II is now being produced by Biosoft. The specificity was determined by enzyme immunoassay and it was shown to recognise both type I and type II herpes simplex viruses equally well. The antibody is derived from the fusion of an SP20 mouse myeloma cell with BALB/c mouse splenocytes which have been immunized with strain G virus. The antibody should be stored at either 4°C or -20°C and repeated warmings above 4°C should be avoided. It can be used for research on the herpes virus either using indirect immunofluorescence or enzyme immunoassay.

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● Sera-Lab have extended their series of monoclonal anti-fibronectin markers (MAS 073) which appear to be specific for human fibronectin. The other anti-fibronectin antibody (MAS 037), while having been derived from a hybridoma formed by fusion of a mouse myeloma cell with a spleen cell from a mouse immunized against human muscle myoblasts, is not species-specific and cross-reacts with rat and chicken muscle cells.

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● Two monoclonal antibodies against tubulin, YL1/2 and YOL1/34 (cat MAS 077 and MAS 078), have been developed by Accurate Chemical. Both antibodies were raised using yeast tubulin as antigen and give immunofluorescent staining of microtubules in cells such as yeast and mammalian tissue culture cells.

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● A full line of monoclonal antibodies for the detection and isolation of defined antigens is now available from Biotech Research Laboratories. The range includes anti-human and rabbit cell surface and histocompatibility antigens, anti-aflatoxin; anti-cytomegalovirus antigens; anti-human complement components; anti-Epstein-Barr virus antigens; anti-herpes simplex virus I, II, and I plus II; anti-human transferrin, and anti-human immunoglobulins.

Circle No. 124 on Reader Service Card

● Hybritech has released its first Tandem-V (visual) monoclonal antibody immunoenzymetric assay in Europe, and has expanded its European list of Tandem-E solid phase, two-site monoclonal antibody immunoenzymetric assays (IEMA). The list of assays now includes: Tandem-V (visual) for HCG testing, Tandem-E (IEMA) for HCG, AFP, IgE and ferritin testing; and Tandem-R (IRMA) for IgE, PAP, HCG, ferritin, hGH, TSH and prolactin testing.

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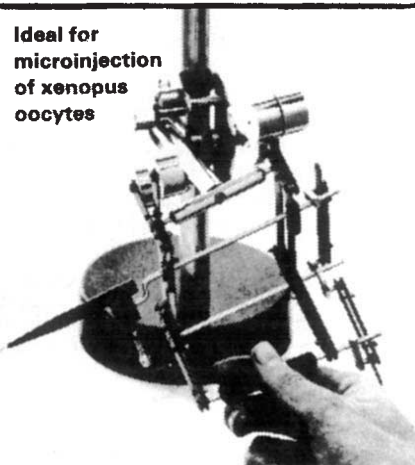
● Becton Dickinson have recently released anti-Leu-11, a new monoclonal antibody which detects NK cells. This antibody reacts with about 15% of human peripheral blood mononuclear cells. Leu-11⁺ cells do not display most typical T cell markers such as Leu-1, Leu-3, or Leu-4. Leu-11⁺ cells isolated by fluorescence-activated cell sorting (FACS) from a peripheral blood mononuclear cell sample contain all the NK function from that preparation. This antibody typically reacts with fewer cells from peripheral blood than anti-Leu-7. By two-colour FACS analysis the following populations can now be demonstrated in normal peripheral blood: Leu-7⁺ 11⁺, Leu-7⁺ 11⁻, Leu-7⁻ 11⁺, and Leu-7⁻ 11⁻. Anti-Leu-11 is available as a purified antibody for cytotoxicity testing (anti-Leu-11b, an IgM antibody) and as a fluorescein conjugate (anti-Leu-11a, fluorescein isothiocyanate conjugated for fluorescence studies).

Circle No. 118 on Reader Service Card

● Sanbio from the Netherlands has introduced five monoclonal antibodies for indirect immunoperoxidase staining on formalin-fixed, paraffin-embedded tissues and frozen sections. These monoclonal antibodies are against human keratin, human neurofilament, human chorionic gonadotropin β subunit, human follicle stimulating hormone, and human glia fibrillary acidic protein. The antibodies are supplied as tissue culture medium from antibody-producing cells.

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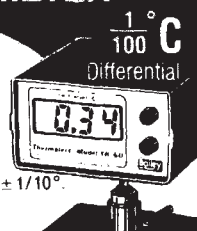


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